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Multi-trophic interactions within the seagrass beds of Banc d'Arguin, Mauritania

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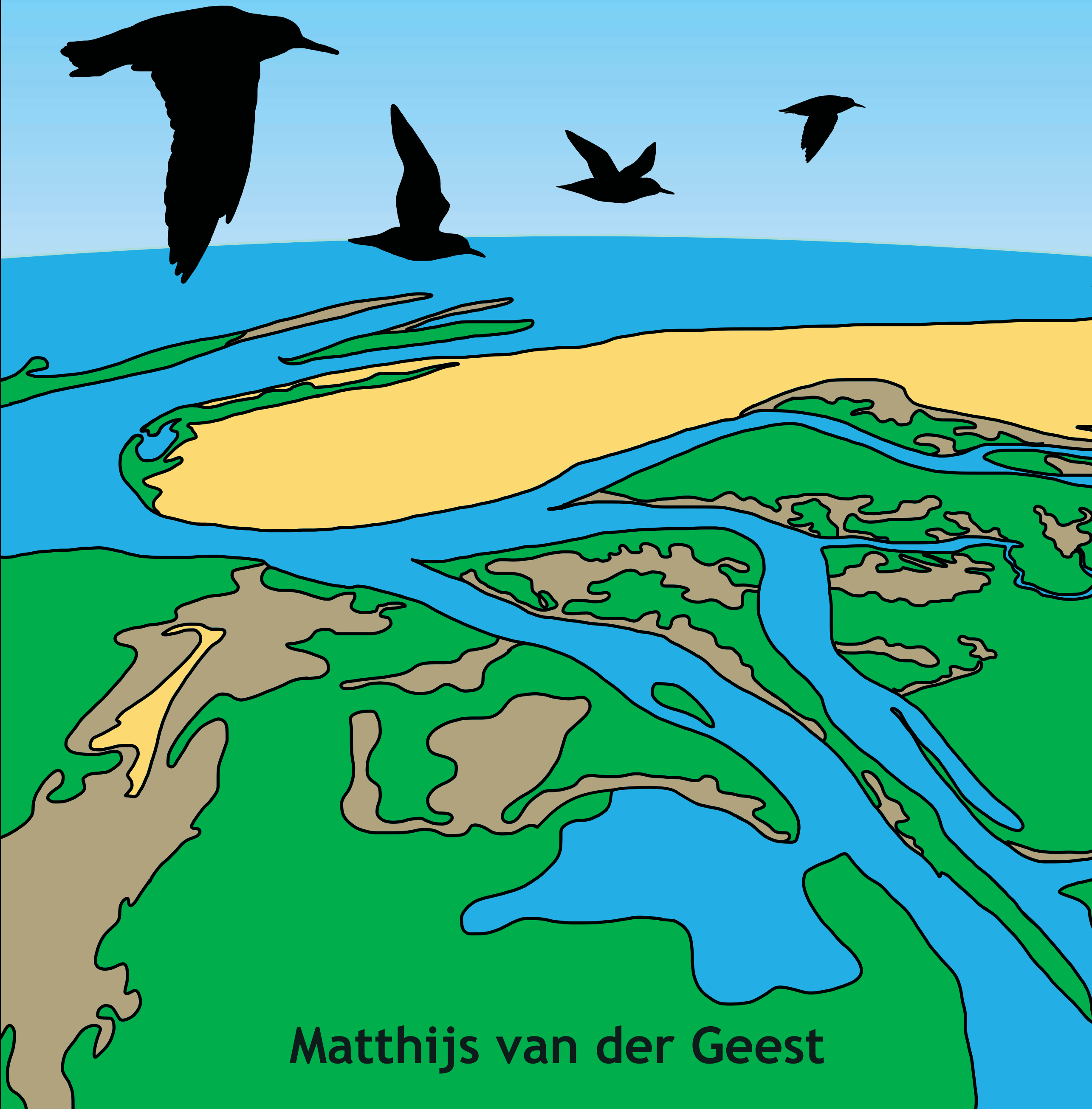
Multi-trophic interactions within the seagrass beds of Banc d'Arguin, Mauritania

A chemosynthesis-based intertidal ecosystem



Matthijs van der Geest

Multi-trophic interactions within the seagrass beds of Banc d'Arguin, Mauritania

A chemosynthesis-based intertidal ecosystem

NIOZ Royal Netherlands Institute for Sea Research

NWO
Netherlands Organisation for Scientific Research
WOTRO Science for Global Development

The work presented in this thesis was conducted at the Department of Marine Ecology (MEE), Royal Netherlands Institute for Sea Research (NIOZ) according to the requirements of the Graduate School of Science (Faculty of Mathematics and Natural Sciencees, University of Groningen). The research was funded by a grant from the Netherlands Organisation for Scientific Research (NWO-WOTRO Integrated Programme, W.01.65.221.00) awarded to T. Piersma. The printing of this thesis was funded by the Royal Netherlands Institute for Sea Research (NIOZ) and the Faculty of Mathematics and Natural Sciences of the University of Groningen.

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Multi-trophic interactions within the seagrass beds of Banc d'Arguin, Mauritania

A chemosynthesis-based intertidal ecosystem

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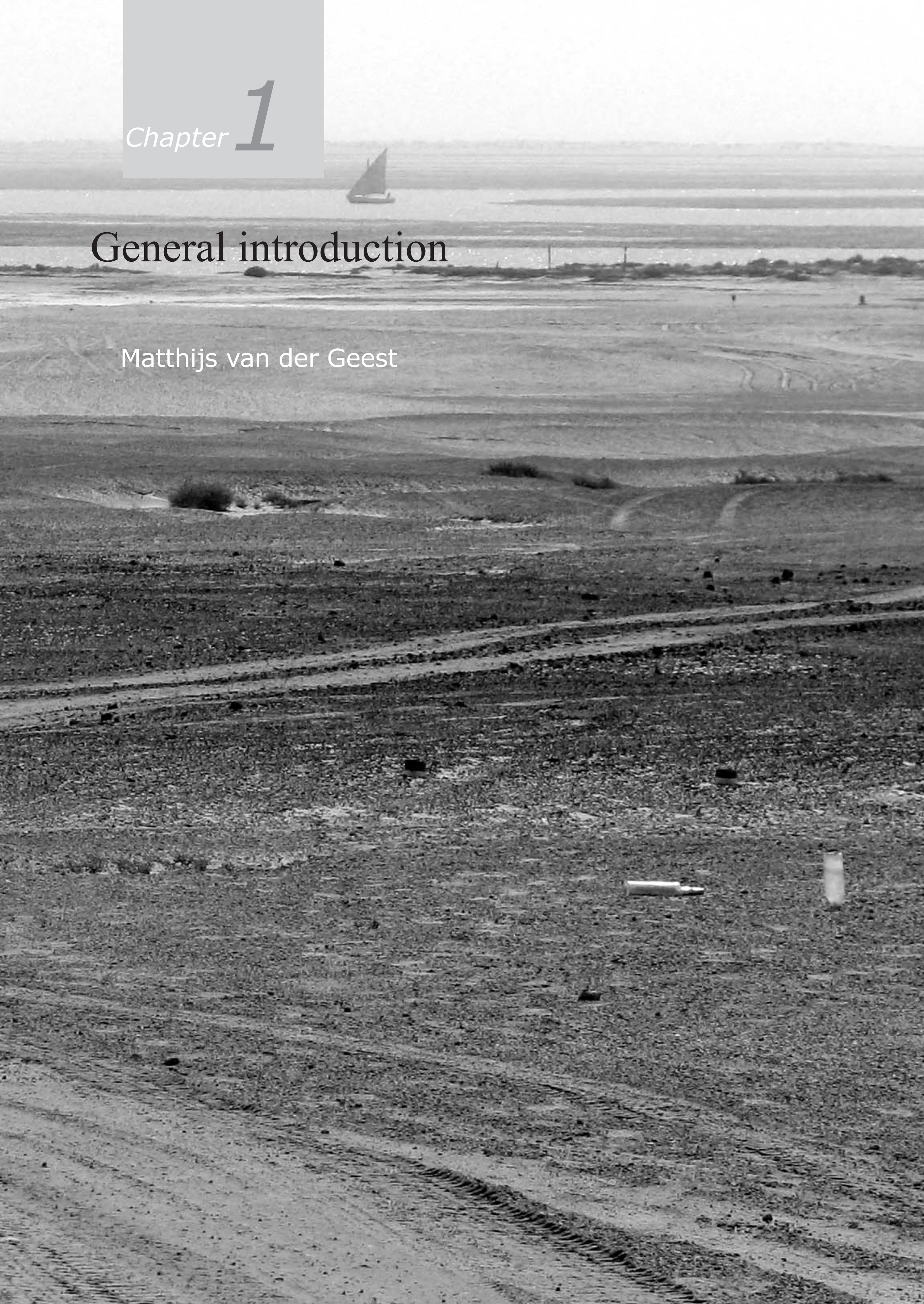
Voor Sem

“In the long history of humankind (and animal kind, too)
those who learned to collaborate and improvise most
effectively have prevailed.”

Charles Darwin (1809-1882)

Contents



Chapter 1

General introduction

Matthijs van der Geest

The distribution and abundance of organisms in space and time, and the interactions within and between species (e.g., predation, competition, mutualism), and between species and their physical and chemical environment, are central to ecology (Begon, Townshend & Harper 2006). Understanding which interactions drive life histories, population dynamics, and community functioning and their response to environmental change is critical for management and preservation of natural communities and ecosystems in a rapidly changing world (Lubchenco *et al.* 1991). This thesis is about the distribution and abundance of organisms living in a tropical seagrass-covered intertidal mudflat, and the physical, chemical, but especially biological features and interactions that determine these distributions and abundances.

The tidal flat ecosystem

A tidal flat can be defined as an area of sea floor that is submerged at high tide and exposed during low tide. Tidal flat occur along tidal channels in bays and lagoons, in estuaries, and along coastal seas and inlets and connect the terrestrial and the marine environments. They range from bare flats to sediments covered with seagrasses or mangroves and can be found in all climate zones, from the Arctic Circle up to the Equator (Eisma 1998).

Intertidal flat systems are known for their high primary productivity, and great abundance of benthic invertebrates (e.g., mollusks, polychaetes, crustaceans) and secondary consumers (e.g. shrimps, crabs, fish, shorebirds) living from this production (Swennen 1976; Pihl & Rosenberg 1982; Jensen & Jensen 1985; Zwarts & Blomert 1992; van der Veer *et al.* 1998; van de Kam *et al.* 2004). Extensive tidal flat systems are quite rare and are usually found at the outflows of major rivers. Indeed, only about two handfuls of coastal intertidal flat systems worldwide support the great majority of northern shorebird migrants, who congregate at such sites during the nonbreeding seasons in large numbers, arriving there from the vast expanses of boreal to high Arctic taiga and tundra (van de Kam *et al.* 2004). In addition, tidal flat systems fulfil great ecological roles as nursery areas for the juvenile stages of offshore fishes and shrimp (Zijlstra 1972; van der Veer, Dapper & Witte 2001), as a habitat for sedentary fishes, their predators and other marine biota and by dissipating wave energy, thus reducing coastal erosion. They are also of great economic value, since many of the organisms that depend on these coastal systems (e.g., lugworms, shellfish, shrimp, fish) are commercially exploited (Beukema 1995; Dijkema 1997; Piersma *et al.* 2001; Lotze 2007).

Many studies have focused on the functioning of intertidal food webs at temperate latitudes, but many fewer ecological studies have been carried out in tropical intertidal ecosystems (Alongi 1990). This lack of knowledge includes the Banc d'Arguin ecosystem off the coast of Mauritania, which has received ecological scrutiny in the 1980s (reviewed by Wolff *et al.* 1993b), but not much since, even though many of the basic questions remained unanswered. This is surprising, since a good understanding of the functioning of this pristine coastal ecosystem, which became a National Park in 1976 and a UNESCO World Heritage Site in 1989, is critical for optimal management and conservation of its natural resources.

The riddle of the Banc d'Arguin

The Banc d'Arguin, Mauritania, northwest Africa, is an area of over 10,000 km^2 of intertidal flats and shallow inshore waters bordering the Sahara desert (Fig. 1.1). With over two million wintering shorebirds, this intertidal system is the most important coastal wintering site along the East Atlantic coast (Trotignon *et al.* 1980; Altenburg *et al.* 1982; Engelmoer *et al.* 1984; Smit & Piersma 1989; Zwarts *et al.* 1990; Zwarts *et al.* 1998). These large numbers of shorebirds are distributed over ca. 500 km^2 of tidal flats only (Wolff & Smit 1990). Not surprisingly, average feeding densities of wintering waders in the intertidal zone of Banc d'Arguin are extremely high when compared to other coastal wintering sites along the East Atlantic coast (Zwarts 1988; Zwarts *et al.* 1990; van Gils *et al.* 2009).

In the past decades some research was carried out to make a beginning of an explanation for the very high densities of wintering shorebirds at Banc d'Arguin. In several studies data was collected on biomass of benthic macrofauna on the intertidal flats, the food of the large majority of these wintering shorebirds (see review in Table 1.1).

These studies established that, contrary to initial expectation, food resources potentially available to shorebirds were relatively low, 7.6–28.6 g ash-free dry mass (AFDM) per m^2, compared to other tidal flat areas in the world where biomass values of 100 g AFDM per m^2 are no exception (Heip 1995; Michaelis & Wolff 2001; Purwoko & Wolff 2008). This value becomes even smaller, ranging between 2.9–8.9 g AFDM per m^2, when we exclude the biomass represented by *Senilia senilis* (see Table 1.1), a large thick-shelled bivalve species that can only be preyed upon by Oystercatchers *Haematopus ostralegus* (Swennen 1990), a relatively uncommon shorebird species on the Banc d'Arguin.

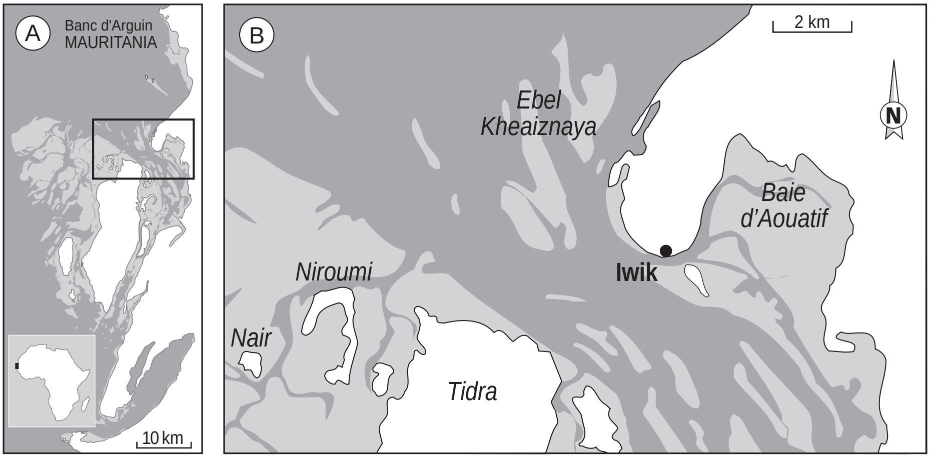


Figure 1.1 (A) Map of the Banc d'Arguin, Mauritania, West-Africa with tidal flats given in light grey, ocean in dark grey and land in white. Note that a small tidal flat area (ca. 65 km^2) around Ile d'Arguin in the north of the Banc d'Arguin ecosystem is not presented. (B) Our study area, near the coastal village Iwik (notably the tidal flats at Abelgh Eiznaya and in the Baie d'Aouatif).

Table 1.1 Macrozoobenthic biomass in g AFDM m^{-2} at the Banc d'Arguin tidal flat area (ca. 500 km^2). Values of benthic biomass have only been included if they are based on surveys covering at least 40% of the entire tidal flat area. Values for the focal bivalve species in this thesis, namely *Senilia senilis* (*S*), *Loripes lucinalis* (*L*) and *Dosinia isocardia* (*D*), are presented separately. NA = not available.

Sampling period	Distribution sampling sites	Bivalvia				Gastropoda	Crustacea	Polychaeta	Other	Total (*S* excluded)	Source
		S	*L*	*D*	Rest						
February 1980	300 km^2	4.7	NA	NA	0.6	0.2	0.3	1.8	0.0	2.9	Piersma (1982)
Feb.–April 1986	300 km^2	8.1	2.6	0.1	0.2	1.9	0.1	3.9	0.1	8.9	Wolff *et al.* (1993a)
September 1988	200 km^2	0.8	1.1	0.2	1.3	0.2	0.5	4.2	0.0	7.5	Wolff & Michaelis (2008)
March–April 2007	200 km^2	20.3	2.6	0.9	1.4	0.1	0.8	2.2	0.3	8.3	Jansen, Folmer & Piersma (2008)

This resulted in the enigmatic situation that over two million wintering shorebirds seemed to depend on relatively low standing stocks of benthic invertebrate food occurring in a relatively small tidal flat area. Piersma and Engelmoer (1982) calculated that, if no regrowth or settlement of benthic animals occurred, the population of wintering shorebirds at Banc d'Arguin would be able to deplete the total benthic biomass in the two months before migration. This implies that production rates by macrobenthic invertebrates must be sufficiently high, but these are unaccounted for (Piersma 1982; Wolff & Smit 1990; Wolff 1991; Wolff *et al.* 1993b; Michaelis & Wolff 2001).

Although the offshore coast of Mauritania is defined as an upwelling area, the clear inshore waters covering the intertidal flats of Banc d'Arguin are believed to be nutrient-limited with slow rates of primary production by phytoplankton and benthic microalgae (Sevrin-Reyssac 1984; De Jong *et al.* 1991; Sevrin-Reyssac 1993; Wolff *et al.* 1993b; Michaelis & Wolff 2001). As the large standing stock of seagrass biomass is considered too refractory to be of any use for most macrobenthic species living at Banc d'Arguin (Wolff *et al.* 1993b; Honkoop *et al.* 2008), there is at first sight no known energy source that could account for the supposedly high secondary production rates by macrobenthic invertebrates.

A novel chemoautotrophic pathway

The question of the presumably high secondary production rates in a nutrient-poor environment might perhaps be explained by the fact that some of the benthic marine invertebrates living at the Banc d'Arguin potentially make use of sugars provided by symbiotic chemoautotrophic bacteria. Chemoautotrophic symbiosis was first described in invertebrates living in deep-sea hydrothermal vents (Cavanaugh *et al.* 1981; Felbeck, Childress & Somero 1981), but it has been described to also occur in marine invertebrates inhabiting more shallow marine habitats, including seagrass beds (e.g., Cavanaugh 1983; Fisher & Hand 1984). A common feature of these habitats is the presence of an interface between a sulphide-generating zone (anoxic) and sediment pore-water with sufficient oxygen to sustain the host animal. In this nutritional association, the invertebrate host favours bacterial chemosynthesis by facilitating the supply of sulphide, CO_2 and oxygen. In exchange, the bacterial symbionts obtain energy by oxidizing sulphide, which is used to fix inorganic carbon into sugars that fuel their own energetic needs, in addition to that of their host (Stewart, Newton & Cavanaugh 2005).

The intertidal flats at Banc d'Arguin are densely covered by seagrass (mainly *Zostera noltii* Hornemann; Wolff & Smit 1990). A large portion of the produced seagrass biomass remains trapped in the seagrass beds and is eventually deposited on the bottom as detritus (Hemminga & Nieuwenhuize 1991). An important fraction of organic matter is decomposed anaerobically by sulphate-reducing bacteria that use the abundant sulphate (SO_4^{2-}) in seawater as an electron acceptor instead of oxygen and produce sulphide (H_2S) as a metabolic end product (Jørgensen 1982). Sulphide is toxic to many organisms as its lipid solubility enables it to freely penetrate biological membranes, eventually slowing down the functioning of mitochondria and the production of ATP (Bagarinao 1992). However, by indirectly stimulating sulphide production through high organic matter input and by providing oxygen through radial oxygen release from the roots, the seagrass meadows of Banc d'Arguin may provide an ideal habitat for benthic invertebrates that live in symbiosis with sulphide-oxidizing endosymbiotic bacteria, as demonstrated in Fig. 1.2. Such chemoautotrophic metabolism could represent an additional metabolic pathway, an attribute of the Banc d'Arguin ecosystem that has hitherto not been recognized. We hypothesize that the presumed high secondary production rates in a seemingly energy-poor environment may be explained by some of the benthic marine invertebrates making use of food provided by endosymbiotic chemoautotrophic bacteria.

Lucinidae: a special family of bivalves

Bivalves belonging to the family of Lucinidae can be very prominent in seagrass beds, because of their likely obligate symbiosis with sulphide-oxidizing bacteria housed in the gills from which they derive much of their nutrition (Distel 1998; Johnson, Fernandez & Pergent 2002; Taylor & Glover 2006). Previous benthic surveys carried out on the seagrass-covered tidal flats of Banc d'Arguin, indeed revealed that one of the most abundant benthic invertebrates in terms of biomass and numbers was *Loripes lucinalis* (Lamarck, 1818; in earlier publications denoted by the name *Loripes lacteus*), a bivalve belonging to the family of Lucinidae (Table 1.1).

To investigate whether *L. lucinalis* harbours chemoautotrophic sulphide-oxidizing bacteria within their gills, we did a preliminary comparative 16S rRNA gene sequence analysis on DNA extracted from their gills (see box A). This revealed the presence of a dominant bacterial phylotype-related to the sulphide-oxidizing symbionts of other lucinid bivalves (Fig. A.1), with comparative analyses of gene sequences encoding the APS reductase α subunit (aprA) and ribulose-1,5-bisphosphate carboxylase oxygenase large subunit (*cbb*L) indicating that this dominant bacterial symbiont can indeed perform sulphide oxidation (Fig. AI.2) and autotrophy (Fig. A.3).

Aims and outline of this thesis

This thesis focuses on the ecological importance of chemoautotrophic symbiosis in the tropical intertidal seagrass beds of Banc d'Arguin (Mauritania) dominated by *Zostera noltii* (dwarf eelgrass). Using this ecosystem as a model, the primary objective of this thesis is to obtain insights concerning the flux of chemoautotrophic fixed carbon in the seagrass beds of Banc d'Arguin, which hitherto were believed to act as a sink of organic carbon. Secondly, we aim to reveal the organizing principles (predation, competition, mutualism) that mould the tropical seagrass-communities. As a model predator the red knot *Calidris canutus canutus* is chosen, the most abundant molluscivore shorebird wintering at Banc d'Arguin

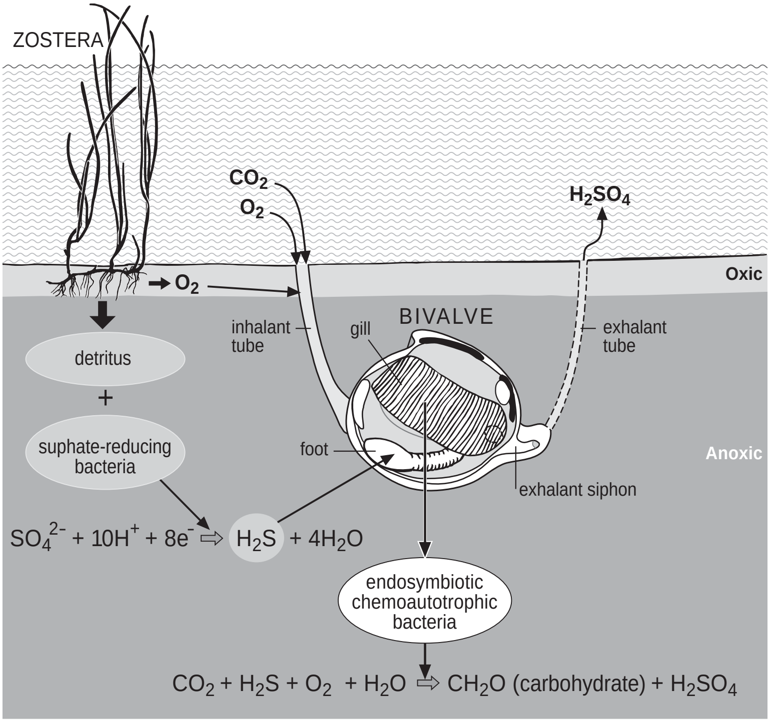


Figure 1.2 Schematic representation of below-ground carbon fluxes in a *Zostera*-dominated intertidal system like Banc d'Arguin. Sulphate-reducing bacteria produce hydrogen sulphide (H_2S) when decomposing *Zostera* detritus, which is absorbed from pore-water by the foot of a lucinid bivalve (here used as an example of a chemoautotrophic endosymbiont-bearing marine benthic invertebrate) and transported to its gills. Oxygen and CO_2 from pore-water and the overlaying water is absorbed by the inhalant tube and also transported towards the gills of the lucinid bivalve. Chemoautotrophic endosymbiotic bacteria located in the gills of the lucinid bivalve oxidize H_2S and use some of the released energy to fix CO_2 in the Calvin cycle. This symbiotically fixed carbon contributes to the growth of both the endosymbiotic bacteria and the lucinid host, of which the latter may eventually be consumed by higher organisms.

(Zwarts *et al.* 1998) for whom the numerically dominant lucinid bivalve *Loripes lucinalis* may form an important food source. Its habitat of ingesting its hard-shelled prey whole and excreting ingestible shell fragments by defecation, allows a precise methodology that enables the quantitative reconstruction of diet composition through faecal analysis (Dekinga & Piersma 1993; Onrust *et al.* 2013). Next, we aim to investigate life-history characteristics (nutrition, growth, reproductive investment and timing of spawning) and the biotic and abiotic factors that may regulate population dynamics of chemoautotrophic endosymbiont-bearing *Loripes lucinalis*. Finally, we aim to provide the Mauritanian authorities with a better understanding of the importance of seagrass food-web interactions, to continue effective management of these valuable natural resources.

Loripes lucinalis (hereafter *Loripes*) is known be a facultative mixotroph partly feeding on sugars provided by sulphide-oxidizing chemoautotrophic gill-bacteria, but also consuming suspended particulate organic matter (POM). To what extent this ability to shift diet contributes to growth, reproductive output and survival in *Loripes* is empirically studied in Chapter 2. Environmental factors that may regulate feeding behaviour and implications of diet shifts for population dynamics and subsequent seagrass community functioning are also discussed in this chapter.

Seagrass beds tend to accumulate organic matter, and so it is expected that seagrass beds would build up toxic sulphides and hence have a limited productivity and diversity. Using a meta-analysis, a field study and a laboratory experiment, we tested the hypothesis that a three-stage symbiosis between seagrasses, associated burrowing lucinid bivalves, and their sulphide-oxidizing gill-bacteria releases the sulphide stress for seagrasses (Chapter 3).

Mark-recapture experiments are often used to estimate growth rates in bivalves. Yet, traditional marking methods (e.g., surface filing, edge notching, painting labels, tagging) involve the physical handling of the shells and removal from their natural habitat which may lead to disrupted shell growth. This may especially be true for fragile deeply burrowed bivalves like *Loripes*. In Chapter 4, we investigated the suitability of calcein as an *in situ* fluorescent growth marker in *Loripes*. The great advantage of such an *in situ* marking method would be that physical stress is minimized because individuals are not touched or moved during the process, which should provide more natural growth rate estimates.

Predators often play key roles in the structuring and organization of ecological communities. By alleviating interspecific competition among prey, predators may promote biodiversity and prey growth rates (i.e., productivity). The cascading top-down role that the most abundant molluscivore shorebird, the red knot, may play on growth rates of their lucinid prey, is experimentally studied in Chapter 5 by locally excluding knots from their prey using exclosures and using the *in situ* calcein-marking technique developed in the previous chapter to estimate lucinid growth rates inside and outside the exclosures. The depletion trajectories of the two bivalve prey species *Loripes lucinalis* and *Dosinia isocardia* (Dunker, 1845; in earlier publications denoted by the name *Dosinia hepatica*), that together make up 85% of all mollusks that are ingestible by knots (Honkoop *et al.* 2008), will tell us whether knots are generalist or specialist predators on these preys.

In Chapter 6 we test the seasonal depletion hypothesis, which states that benthic biomass at Banc d'Arguin builds up in summer to reach a peak value in September and subsequently is grazed down during the wintering season by shorebirds. We do so by quantifying monthly changes in mollusk biomass over a full year in a seagrass bed, intensively used by wintering red knots.

In Chapter 7 we explore the extent to which timing of reproduction in *Loripes* could be moulded by seasonal predation by size-selective knots wintering at Banc d'Arguin. To do so, we used *in situ* collected data to determine size- and season-specific predation and growth rates of *Loripes* and examined whether the reported timing of spawning matched with the quantified optimal time of spawning that minimizes post-settlement offspring predation rate by knots until size at first reproduction.

Next, using an 8-year field study on food abundance, diet choice, survival rate and population size in our focal molluscivore predator, the red knot, in its non-breeding area at Banc d'Arguin, we explore how much knot population dynamics depend on the presence of either *Loripes lucinalis* or *Dosinia isocardia* (Chapter 8).

Density-dependent processes occur when population growth rates are regulated by the density of a population. By thinning prey that are subject to inter- or intraspecific competition, predators could boost food supplies by their own predatory acts. Density-dependent processes thus have cascading trophic effects and contribute to the structuring of interaction webs. In Chapter 9, we manipulated local densities of chemosynthetic and suspension-feeding benthic communities in contrasting intertidal habitats (sandy bare sediments versus seagrass-covered mud) to explore density-effects on *in situ* growth rates of three numerically abundant bivalve species (*Loripes lucinalis*, *Dosinia isocardia* and *Senilia senilis*) during autumn-winter (the season of slow growth) and spring–summer (the season of fast growth). In Chapter 10, we review and discuss the main conclusions from the previous chapters and test whether production rates of the numerically abundant bivalve species are sufficient to sustain the molluscivore red knot population at Banc d'Arguin. The main aims are to establish the significance of our results to our understanding of the ecological functioning of the tropical intertidal flats of Banc d'Arguin, with a focus on the multi-trophic interplay between different ecological groups, and to define directions for further research.

Box A

Molecular evidence for sulphide-oxidizing endosymbiosis in *Loripes lucinalis*

Matthijs van der Geest & Judith van Bleijswijk

The lucinid bivalve *Loripes lucinalis* is predominant in the reduced seagrass sediments of Banc d'Arguin, Mauritania. To assess its supposed symbiosis with autotrophic sulphide-oxidizing gill-bacteria, total DNA was extracted from the gills of *L. lucinalis*. The PCR fragments corresponding to the genes encoding 16S rRNA, APS reductase α subunit (aprA, an enzyme involved in the sulphide oxidation pathway) and ribulose-1,5-bisphosphate carboxylase oxygenase (RubisCO) large subunit (an enzyme of the Calvin-Benson cycle of autotrophic CO_2 fixation) were amplified and sequenced. For the 16S rRNA genes, two phylotypes were revealed, both belonging to *Gammaproteobacteria*; one belongs to the cluster of thiotrophic lucinid symbionts and the other belongs to the genus of *Shewanella*, a free-living facultative anaerobic gammaproteobacterium (Fig. A.1). Comparative analysis of gene sequences encoding aprA and RubisCO, support the hypothesis that the dominant gill-symbiont of *L. lucinalis* can perform sulphide oxidation (Fig. A.2) and autotrophic activity (Fig. A.3).

Acknowledgements

We thank Anneke Bol for accurately doing the DNA extractions, all PCRs on functional genes and preparations for sequencing. Julie Ogier and Dennis Mosk are thanked for doing PCR-DGGE on ribosomal RNA genes and Harry Witte for optimising lab equipment.

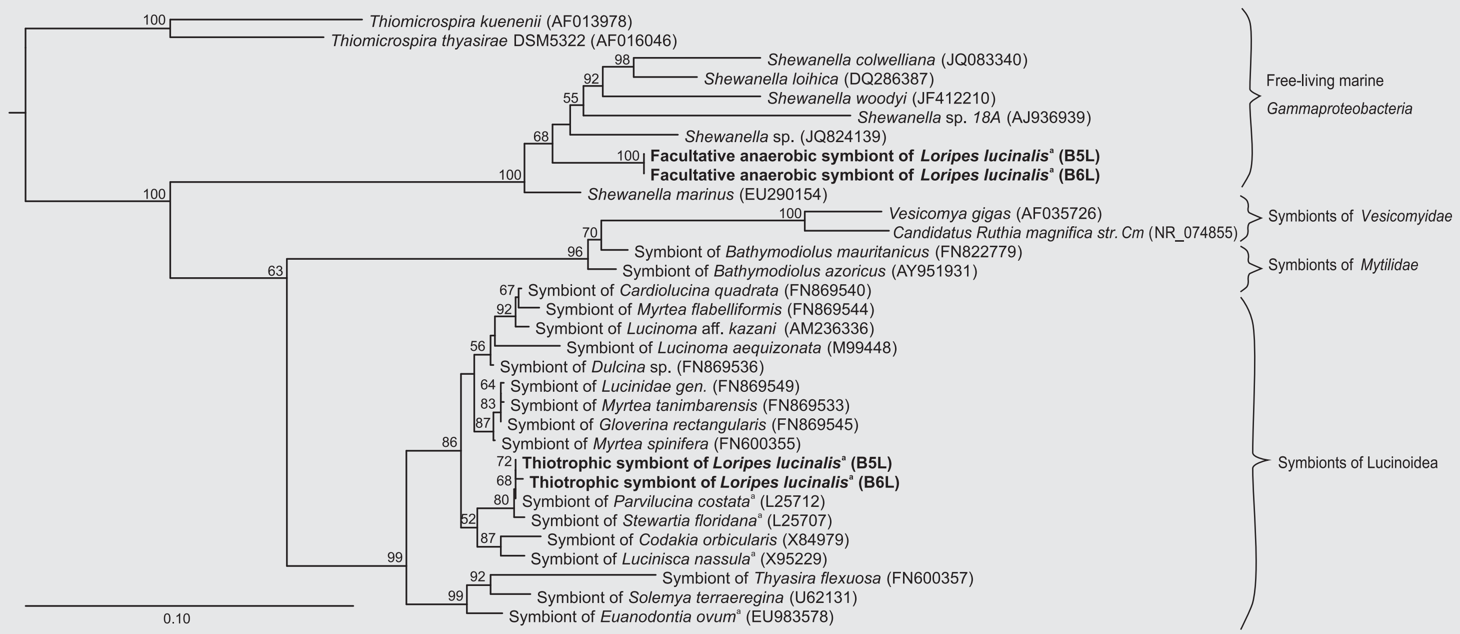


Figure A.1 RaxML Maximum-likelihood tree (L = -7550.02) with 16S rRNA sequences retrieved from DGGE profiles of gill tissues of two *Loripes lucinalis* individuals (B5L, B6L, in bold). The reference tree with 29 species was calculated based on 1429 bases using a bacterial position variability filter by parsimony and rapid bootstrap analyses with 100 runs (only values >50% are shown). Shorter sequences were added to the reference tree using the ARB Parsimony tool: sequence L25707 with 1359 bases, AJ936939 and M99448 with 1340 bases, KC520579 with 889 bases and our four sequences of *Loripes lucinalis* symbionts with 559 bases. Two of these sequences shared 497 out of 559 bases with JQ824139, *Shewanella* sp. and two shared 559/559 and 558/559 bases with L25712, a thiotrophic symbiont of *Parvilucina costata*. Scale bar represents 10% estimated nucleotide substitution. [a]Formerly *Loripes lacteus* (*Loripes lucinalis*), *Codakia costata* (*Parvilucina costata*), *Lucina floridana* (*Stewartia floridana*), *Lucina nassula* (*Lucinisca nassula*), *Anodontia ovum* (*Euanodontia ovum*).

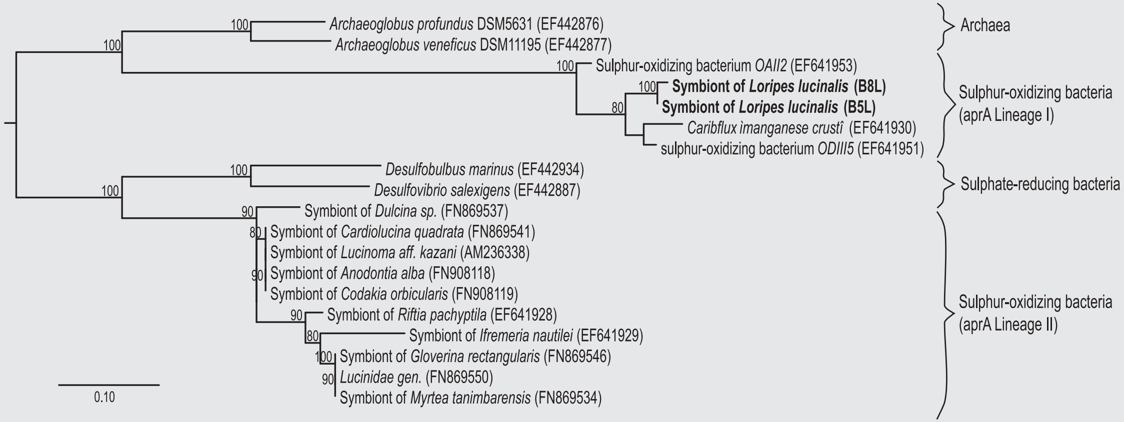


Figure A.2 RaxML maximum-likelihood tree (L = -1158.06) with the APS reductase α subunit of two individuals of *Loripes lucinalis* (B5L, B8L, in bold). The tree was calculated based on 100 amino acids with rapid bootstrap analyses over 100 runs (only values >50% are shown). Both sequences from *L. lucinalis* fall in the Lineage I of *aprA* in the sulphur-oxidizing bacteria clade. The sequences of *Loripes lucinalis* symbionts shared 92/100 and 91/100 amino acids with a sulphur-oxidizing bacterium *ODIII5* EF641951. Scale bar represents 10% estimated amino acid substitution.

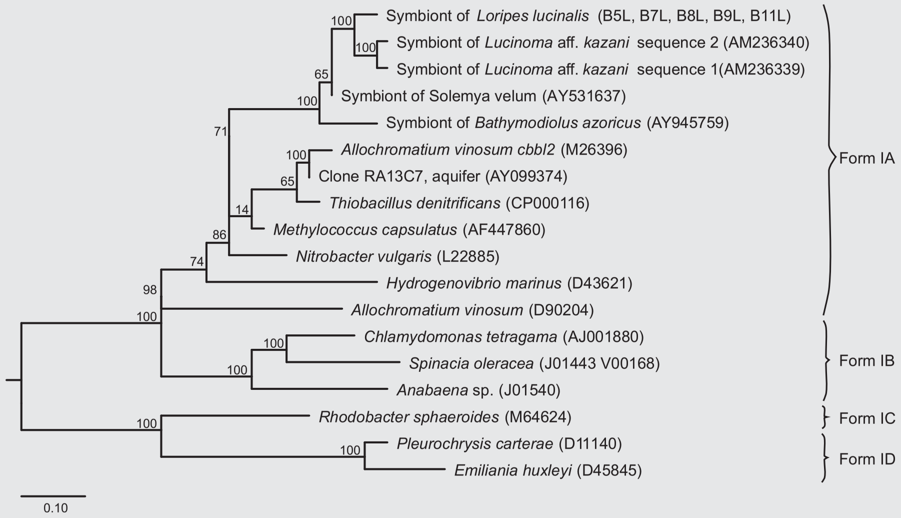


Figure A.3 RaxML Maximum-likelihood tree (L = -3198.68) with the RubisCO large-subunit of five individuals of *Loripes lucinalis* (B5L, B7L, B8L, B9L, B11L in bold). The tree was calculated based on 239 amino acids with rapid bootstrap analyses over 100 runs. All sequences from *L. lucinalis* cluster together within the Form IA cluster. Symbionts shared 227 out of 239 amino acids with the thiotrophic symbionts of *Solemya velum* and 76 out of 83 amino acids with the thiotrophic symbionts of *Lucinoma* aff. *kazani*.

Chapter 2

Nutritional and reproductive strategies in a chemosymbiotic bivalve living in a tropical intertidal seagrass bed

Matthijs van der Geest, Amadou Abderahmane Sall,
Sidi Ould Ely, Reindert W. Nauta, Jan. A. van Gils &
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Abstract Sulphide-oxidizing endosymbiont-bearing bivalves often dominate the infauna of seagrass-covered sediments, where they control sulphide levels and contribute to carbon cycling by feeding on chemosynthetically fixed carbon and on suspended particulate organic matter (POM). Previous studies from temperate habitats suggest that POM-availability may regulate growth and reproduction, since POM may be of greater nutritional value than the material provided by bacterial endosymbionts. To examine if changes in diet correlate with body condition and reproductive activity, for a tropical study site we studied seasonal patterns in somatic and gonadal investment and gametogenic development in relation to nutrition in the endosymbiont-bearing bivalve *Loripes lucinalis* in the seagrass-covered intertidal flats at Banc d'Arguin, Mauritania. Carbon stable isotope analysis revealed clear seasonal cycles in the relative heterotrophic contribution to the diet of *Loripes*, with mean monthly values ranging from 16% in March to 31% in September. Seasonality was also observed for size-corrected body and somatic mass, both increasing from March to October, suggesting food limitation during winter. In contrast, *Loripes* exhibits a semi-annual reproductive cycle characterized by major spawning events between January and February and between July and August. Growth and gametogenic development seem to require supplemental heterotrophic food, except during spring and early summer. Thus, the ability to shift to heterotrophic feeding contributes to growth, reproductive output and survival in *Loripes*, with downstream effects on population dynamics and seagrass functioning.

Introduction

Nutritional associations between intracellular sulphide-oxidizing bacteria and marine invertebrates were discovered in deep-sea hydrothermal vents by Felbeck *et al.* (1981). A few years later they were also discovered in more accessible habitats, such as shallow-water sediments (e.g., Cavanaugh 1983; Fisher & Hand 1984; Schweimanns & Felbeck 1985). In this association, the invertebrate host favours bacterial chemosynthesis by facilitating the supply of sulphide, CO_2 and oxygen. In exchange, the bacterial symbionts obtain energy by oxidizing sulphide, which is used to fix inorganic carbon into sugars that fuel their own energetic needs, in addition to that of their host (Stewart, Newton & Cavanaugh 2005).

Among chemosymbiotic organisms, bivalves of the Lucinidae family are the most species-rich group. Lucinids occupy the greatest variety of habitats and are geographically the most widespread (60°N–55°S) (Taylor & Glover 2006). Their sulphide-oxidizing symbiosis is probably obligate, and this may explain why lucinids often dominate the infauna of sulphide-rich anoxic sediments of seagrass meadows (van der Heide *et al.* 2012). Here, the bacteria-lucinid bivalve association forms a main component of the intertidal benthic community, with top-down effects on sediment biogeochemistry and ecosystem functioning (van Gils *et al.* 2012), by reducing toxic sulphide levels and thereby enhancing seagrass production (van der Heide *et al.* 2012), and bottom-up effects by converting CO_2 directly into consumer biomass (van Gils *et al.* 2012). Despite their important ecological functions in seagrass ecosystems, general principles of the reproductive biology and energetics of lucinids and other endosymbiont-bearing bivalves remain poorly understood (reviewed in Le Pennec & Beninger 2000).

In bivalves, as in other organisms, the timing and duration of reproductive activity and spawning are generally believed to be determined by an interaction between exogenous (environmental) and endogenous (genetic and physiological) factors (reviewed in Sastry 1979). Two of the main exogenous factors commonly cited as reproductive timing cues in heterotrophic bivalves are temperature and particulate organic food availability (Sastry 1979; MacDonald & Thompson 1986; Santos *et al.* 2011).

Like most endosymbiont-bearing bivalves, lucinid bivalves have a mixotrophic diet, feeding on endosymbiotic bacteria and/or on suspended particulate organic matter (POM) from the water column (Le Pennec *et al.* 1988; Cary, Vetter & Felbeck 1989; Duplessis *et al.* 2004; Rossi *et al.* 2013). This flexible feeding mode may help them survive in environments where the concentration of particulate food, sulphide and oxygen varies in space and/or in time (Dufour & Felbeck 2006; van Gils *et al.* 2012; Rossi *et al.* 2013). Although the nutritional role of heterotrophy in endosymbiont-bearing bivalves is poorly understood (Pile & Young 1999), there is some indirect evidence from temperate littoral and deep-sea habitats that seasonality in POM-availability may regulate growth rate and reproductive activity in endosymbiont-bearing bivalves (Le Pennec *et al.* 1995; Pile & Young 1999; Le Pennec & Beninger 2000; Dixon *et al.* 2006; Tyler *et al.* 2007). POM is considered to be more nutritionally and energetically rich than food provided by endosymbiotic bacteria (Le Pennec *et al.* 1995; Pile & Young 1999; van Gils *et al.* 2012). The synchronization of energetically costly gamete production and breeding to periods of increased POM availability may therefore be an evolutionary adaptation: adults can profit from supplementary nutrition, which will increase their reproductive output and survival prospects, while their offspring can profit from optimal food conditions when born, which will increase their survival prospects.

Although cyclic environmental events including fluctuations in temperature, photoperiod, tidal rhythm, salinity and particulate food availability are present in virtually all littoral habitats (Le Pennec & Beninger 2000), they are usually considered little pronounced in the tropics. This would lead to more continuous gametogenic activity and spawning in marine invertebrates living there (Sastry 1979; Fournier 1992; Pouvreau *et al.* 2000). If variability in POM availability indeed regulates the reproductive cycle of chemosymbiotic bivalves, then one would predict less pronounced seasonality in feeding behaviour and as a result more continuous gametogenic activity and breeding in tropical chemosymbiotic bivalves.

Carbon stable isotope compositions have proven to be a useful tool to study the main sources of carbon assimilated by chemosymbiotic bivalves (Spiro *et al.* 1986; Conway, Capuzzo & Fry 1989; Dando & Spiro 1993; Rossi *et al.* 2013). Contrary to gut content analyses, carbon stable isotope analyses allow determination of food sources actually assimilated in the tissues of consumers over time, properly reflecting their trophodynamics depending on food source availability (Fry 2006). Fractionation of carbon stable isotopes ($\delta^{13}C$) is limited to about 1‰ per trophic level (Peterson & Fry 1987), and thus it can be used to identify carbon sources in the diet of organisms (Fry 2006).

The littoral endosymbiont-bearing lucinid bivalve *Loripes lucinalis* (Lamarck, 1818; now considered synonymous with *L. lacteus*, Taylor 2012) is a mixotrophic species (Le Pennec *et al.* 1988; Johnson, Diouris & Le Pennec 1994) covering a wide geographical range (from 55°N to 19°N) from the British Isles in the north to Mauritania in the south. Previous studies investigating the reproductive cycle in a temperate littoral *Loripes lucinalis* population showed clear seasonality in gametogenic activity and breeding with spawning events occurring in May and to a lesser extent between November and December, both of which were preceded by phytoplankton blooms (Le Pennec *et al.* 1995).

We here examine the degree of ‘tropical seasonality’ in diet, growth (measured as an increase in size-corrected body mass), somatic and gonadal investment, and gametogenic development of *Loripes lucinalis* (hereafter *Loripes*) at the southern border of its distributional range, the tropical seagrass-covered intertidal flats of Banc d’Arguin, Mauritania (20ºN). Previous studies have indicated that at Banc d’Arguin, benthic diatoms are the primary POM source (Sevrin-Reyssac 1984; Wolff *et al.* 1993b; Honkoop *et al.* 2008). Living in the sediment-water interface where physical forces due to wind and tidal action can be high, these benthic diatoms often become suspended in the water column, making them also available to suspension-feeding organisms (de Jonge & van Beusekom 1995). The inclusion of POM from a local source (i.e., resuspended benthic diatoms) to the diet of *Loripes* would imply tight energy cycling within the system. Assuming that benthic POM availability is rather constant at tropical latitudes, we predict little seasonality in feeding behaviour and as a result continuous gametogenic activity and breeding in chemosymbiotic *Loripes* from tropical Banc d'Arguin. We will test this prediction by analyzing: (1) degree of heterotrophic feeding by following changes in *Loripes* carbon isotopic signatures throughout the year; (2) degree of seasonality in reproductive activity by assessing the seasonal patterns in gonadal mass and gametogenic development. Furthermore, we investigated reproductive strategies in *Loripes* by analyzing mass allocation to growth and reproduction by following changes in body, somatic and gonadal cycles throughout the year. In addition, we investigated if changing environmental conditions (i.e., temperature, precipitation, wind speed) may regulate the degree of heterotrophic feeding, growth and reproductive activity in *Loripes* and if temporal patterns in heterotrophic feeding correlate with periods of growth and reproductive activity in *Loripes*.

Materials and methods

Study site

The study area, located near the coastal village Iwik (19°52.42´N, 16°18.50´W), is an accessible part of the intertidal area of the nationally protected Banc d'Arguin off the coast of Mauritania. This ecosystem is characterized by tidal flats densely covered by seagrass (mainly *Zostera noltii* Hornem.; Wolff and Smit, 1990). The intertidal flat area is a major wintering site for migrating shorebirds and is also renowned as a nursery area for fish (Altenburg *et al.* 1982; Jager 1993). Being adjacent to the Sahara desert, the Banc d'Arguin does not receive freshwater inflow from rivers, while precipitation is limited to occasional thunderstorms that occur at irregular intervals, sometimes several years apart (Wolff & Smit 1990). In the Iwik region, seawater temperature varies between 20°C in winter and 30°C in summer (Wolff & Smit 1990). Winds are rather strong year-round, but with highest monthly average wind speeds (more than 8 m s^{-1}) in April–July and relatively low wind speeds (less than 7 m s^{-1}) in October–January (Wolff & Smit 1990; Dedah 1993). Given the shallow characteristics of the Banc d'Arguin, wind may be a strong factor affecting turbidity and subsequent productivity by benthic microalgae and seagrasses. The tide is semi-diurnal and the tidal range is 1.5–2 m. In the reducing sediments of the intertidal seagrass-covered flats, *Loripes* is the most abundant bivalve species with an average density of 340–780 individuals per m^2 (Honkoop *et al.* 2008) and maximum densities of 4,900 individuals per m^2 (pers. obs. in October 2009). Here, they live within the network of rhizomes of *Zostera noltii* at depths of 0–10 cm, a zone of high sulphide concentrations (van Gils *et al.* 2012).

Environmental parameters

We collected environmental data (sediment and air temperature, precipitation and wind speed) during our study period (October 2009–January 2011) and in the preceding 9 months (January 2009–October 2009). Sediment temperature (°C) at 2 cm depth was recorded every 30 min during most of the study period (from 1 January 2009 to 11 October 2009 and from 14 December 2009 to 7 October 2010) with a logger (Stow-Away® TidbiT®). Mean daily air temperature (°C), total monthly precipitation (mm) and mean monthly wind speed (m s^{-1}) were obtained from the closest weather station at Nouadhibou, 134 km northwest of the study site (via http://www.tutiempo.net). Previous studies showed that average wind speeds at Nouadhibou do not differ significantly from those at our study site near Iwik (Dedah 1993).

Sampling

Loripes was collected at a muddy intertidal seagrass (*Z. noltii*)-covered site in the vicinity of Iwik (N 19˚53.540'N, 16˚18.856'W). The sampling site covered an area of 625 m^2 that remains emerged for 50% of the tidal cycle and can be characterized by muddy sediment with a relatively high percentage of silt/clay (for sediment characteristics at our study site see Appendix I, Section I.1).

Sediment cores were collected once per month from October 2009 to January 2011 to acquire a random sample of *Loripes* across all available size classes. Care was taken to keep a distance of 5 m between monthly sampling sites. Sediment cores (diameter of 15 cm) were taken to a depth of 20 cm and sieved over a 1-mm mesh. From each sieved benthic sample all *Loripes* individuals were sorted within 24 h and preserved in borax-buffered formalin (4%). For each month, a sub-sample of 10–36 individuals was selected haphazardly, covering the entire growth range.

Body mass and reproductive cycle

To identify the reproductive cycle of *Loripes* we applied the method of Johnson *et al.* (1996), who used mass loss in the gonad-digestive gland complex (GD) as an indicator of spawning periods in *Loripes*, because the gonad and digestive gland are intertwined and cannot easily be separated.

For each specimen, shell length (L), shell height (H) and shell thickness (T) was measured with electronic calipers (precision 0.1 mm), after which bivalves were opened and gonad-digestive gland tissue was separated from the remaining somatic mass under a binocular microscope (10×). A smear sample of the gonad tissue was examined microscopically to determine sex and reproductive stage of each individual using a semi quantitative scale modified from Guillou *et al.* (1990):

indifferent: no gonad tissue visible. This has two possible explanations: adults with recuperating gonads after spawning events (1) or immature juveniles (2).

developing 1: gonad tissues visible, but it is very difficult to distinguish sexes. No mature elements are present.

developing 2: gonad tissues are evident and sexes can be distinguished. Gametes are abundant, and pedunculate oocytes are present.

ripe: gonad tissue with spermatozoa grouped into rings or spherical oocytes with a thick envelope. Spawning is imminent.

spent: gonads are empty and thin. Coexistence of cells being reabsorbed and mature cells.

After microscopic analysis the smear sample was carefully added to the remaining GD tissues and dry mass (DM) was determined to the nearest 0.01 mg for both GD and somatic tissues by drying for 48 hr at 60°C. To follow investment in somatic and GD mass, the somatic mass index (SMI) and the gonadal mass index (GMI) were estimated as the dry mass of the soma, or GD, respectively, divided by a proxy of shell volume calculated as L·H·T to allow comparison between animals of different sizes. Body mass indices are generally considered to indicate the seasonally changing nutritional status of bivalves (reviewed in Crosby & Gale 1990). In order to determine body condition, the body mass index (BMI) was calculated as the total body DM (soma + GD) divided by L·H·T.

Immature animals have smaller gonads than mature animals and the cycles of their gonad weights and gametogenesis etc., may well differ (Grant & Tyler 1983). Immature animals should thus be excluded from analyses of the timing of reproductive activity. To determine size at first maturity we plotted the proportion of sexed animals (e.g., those animals with reproductive cells) against shell height. Subsequently, we excluded *Loripes* smaller than the obtained minimum size at which the majority of *Loripes* could be sexed from further analyses.

Because specimen that have reached size at maturity don't necessarily reproduce, we used the specimens collected during the defined spawning period(s) to identify the minimum shell height at which the majority of the *Loripes* population was in spent stage, here defined as the size at first reproduction.

Carbon stable isotope analysis

For mixotrophic endosymbiont-bearing bivalves, knowledge of both the bacterial and host carbon isotope signatures and the carbon isotopic composition of the more typical suspended POM-based food source is a prerequisite for quantifying the extent of nutritional coupling between the host and its endosymbionts (Conway, Capuzzo & Fry 1989; Rossi *et al.* 2013).

Using the same sampling methodology as described above (see section *Sampling*), we collected *Loripes* specimens at our seagrass-covered intertidal study site in May 2008. The shell height was measured for each individual with calipers (precision 0.1 mm). Each animal was dissected to remove the endosymbiont-bearing gills from the remaining tissues (from the latter the gonad–digestive gland complex was removed). Specimens of *Dosinia isocardia* (Dunker 1845; *Dosinia* hereafter), a venerid bivalve, were also collected at our sampling site and dissected by removing the gut from remaining tissues. All tissue samples were stored frozen (-20˚C) until further analysis.

The carbon isotopic signature of *Dosinia* was used as a proxy for benthic POM (i.e., resuspended benthic diatoms), because these bivalves are benthic suspension feeders that acquire their food through their siphon at the sediment–water interface (Honkoop *et al.* 2008: note that in this paper *D. isocardia* was named *D. hepatica*; recent taxonomic insights have led to this change in nomenclature; R. von Cosel, personal communication). When feeding on heterotrophic food, *Loripes* acquires food through a similar suspension-feeding mode (Rossi *et al.* 2013). Choosing to use *Dosinia* as a proxy rather than sampling particulate organic matter avoided the necessity to correct data for fractionation and yielded values that integrated benthic POM sources.

Although, bivalves are exceedingly proficient at sorting material before it is consumed (reviewed in Ward & Shumway 2004), we assume that *Loripes* and *Dosinia*, which both dominate the molluscan community within the seagrass beds of Banc d'Arguin (Honkoop *et al.* 2008), are utilizing the same size-fractions of benthic POM available at the sediment–water interface. This assumption is supported by van Gils *et al.* (2012) who observed both reduced growth rates in chemosymbiotic *Loripes* and reduced levels of pore-water sulphide when densities of suspension-feeding *Dosinia* increased, suggesting that stronger competition for benthic POM forces *Loripes* to rely on less energetically rich food produced by endosymbiotic bacteria, thus leading to an uptake of sulphide from the surrounding pore-water and a reduction in growth rate.

At our study site, *Dosinia* can reach a maximum shell height of ~20 mm, whereas *Loripes* doesn't grow much larger than 11 mm in shell height (M. van der Geest and J. A. van Gils, *unpublished data*). To correct for potential size-effects on ingested size-fractions of benthic POM and subsequent carbon isotope signature (Rossi *et al.* 2013), we only used *Dosinia* specimens within the size-range of mature *Loripes* (up to 11 mm in shell height) for carbon isotope analysis.

To study the degree of bacterial enrichment, gill tissues of 10 mature *Loripes* specimens were pooled, washed three times in sterile artificial seawater (ASW), and gently homogenized over ice in a Braun homogenizer. Differential centrifugation was used to remove animal cells and resulted in gill material highly enriched in bacterial tissue with respect to the original gill (for further details of the differential centrifugation protocol used we refer to Conway, Capuzzo & Fry 1989). This procedure was repeated for gills from another 10 mature *Loripes* specimens. Microscopic examination of the two bacterial pellets indicated a large reduction in the presence of *Loripes* cell nuclei and other organelles.

To investigate seasonal variability in heterotrophic (i.e., benthic suspension feeding) nutritional contribution, we haphazardly selected a minimum of 3 mature *Loripes* specimens from the monthly benthic samples taken at our study site between October 2009 and January 2011. Again, from each *Loripes* the gills were dissected from remaining tissues as described above, with the only difference that these tissues were preserved in formalin (4%). Whereas freezing is generally considered to be the unique method that does not affect $\delta^{13}C$ values of marine organism (Bosley & Wainright 1999; Kaehler & Pakhomov 2001), tissue fixation in formalin often results in significantly depleted $\delta^{13}C$ values (reviewed in Barrow, Bjorndal & Reich 2008). To correct for possible effects of formalin on *Loripes* $\delta^{13}C$ values, we did an explorative analysis. In August 2010, we sampled *Loripes* (ranging between 8 and 9 mm in shell length) of which we removed the somatic tissue, which was either stored frozen at -20ºC ($N = 8$) or stored in borax-buffered formalin (4%; $N = 3$). Subsequently, $\delta^{13}C$ signatures were determined and a *t*-test was used to compare $\delta^{13}C$ values of somatic tissues from both preservation treatments. This resulted in a significant difference between tissue preservation treatments ($t = 3.83$, df = 7.4, $P = 0.006$), with $\delta^{13}C$ values in *Loripes* tissues preserved in formalin being 0.76‰ (95% CI: 0.30–1.22) more depleted compared to those preserved frozen. We therefore corrected δ^{13}C values for monthly collected *Loripes* specimens that were preserved in formalin by adding 0.76‰ to the obtained δ^{13}C values and used these corrected values for further analyses.

All animal tissues and the two bacterial pellets were freeze-dried for 72 h, homogenized by mortar and pestle and analyzed for carbon stable isotope ratios. The δ^{13}C values were obtained using a Thermo Scientific (Flash 2000) Elemental Analyzer coupled to a Delta V isotope mass spectrometer. The δ^{13}C values for organic carbon are expressed per mil (‰) deviation (δ) from the VPDB standard following the formula: $\delta^{13}C = [(^{13}C/^{12}C)_{\text{sample}}/(^{13}C/^{12}C)_{\text{standard}} - 1] \cdot 1000$. The standard deviation for duplicate runs was ±0.2‰. Explorative analysis showed that δ^{13}C values of either gill ($t = -0.86$, df = 14, $P = 0.40$) or non-gill tissues ($t = -1.62$, df = 14, $P = 0.13$) were not significantly different after treatment of tissue samples with 2 M HCl (to remove inorganic carbon 2 M HCl was added for 12 h at 60°C after which samples were rinsed three times with demineralized water to remove $CaCl_2$ and dried in an oven for 12 h at 60°C). Therefore, we refrained from treating the remaining samples with 2 M HCl prior to carbon stable isotope analysis.

It was assumed that the lucinid host could derive food from two sources, the endosymbiotic bacteria or benthic POM (i.e., resuspended benthic diatoms), similarly to other suspension-feeding bivalves living in the same seagrass habitat (i.e., *Dosinia*). For each month the heterotrophic contribution to the carbon budget of *Loripes* was determined with a two-source mixing model:

$$F = 1 - (\delta^{13}C_{\textit{Loripes}\ \text{non-gill}} - \delta^{13}C_{\textit{Dosinia}}) / (\delta^{13}C_{\text{bacterial pellet}} - \delta^{13}C_{\textit{Dosinia}})$$

where F is the fractional heterotrophic contribution and $\delta^{13}C_{\textit{Loripes}\ \text{non-gill}}$ represents the δ^{13}C value of *Loripes* non-gill tissue corrected for the depletion effect of formalin. Carbon isotope values of the bacterial pellet were assumed to represent the endosymbiont δ^{13}C values, while carbon isotope values of tissues of bivalves feeding on benthic POM (i.e., *Dosinia*) were taken to represent a benthic POM-based food source (Conway, Capuzzo & Fry 1989; Johnson, Diouris & Le Pennec 1994; Rossi *et al.* 2013). Following previous studies (Rossi *et al.* 2013), a trophic enrichment factor of 1‰ was used for the carbon isotope values of the bacterial pellet.

Statistical analysis

We tested for seasonality by using a non-linear mixed effect model with a sinusoidal effect $\beta_0 + \beta_1 \cdot \text{Month} + \text{Season}$. Because explorative data analysis showed that there was a time trend in most of the parameters of interest (BMI, SMI, GMI and percentage of heterotrophic contribution), we accounted for a time trend in all models by including the term $\beta_1 \cdot \text{Month}$, where Month was a continuous variable than ran from 1 to 16. Any seasonal variation was described as $\beta_2 \cdot \text{Msin}(2\pi \cdot (\text{Month-}\beta_3)/\beta_4)$, where parameters β_2, β_3 and β_4 refer to the amplitude of seasonality, the timing of the peak and the frequency of peaks per year.

Visual inspection of the data showed that the BMI, SMI, GMI and gametogenic cycle of males and females followed the same seasonal pattern, so data from both sexes were analyzed together to increase sample size. Using the full model, we tested for the frequency of peaks per year by setting β_4 to 3, 4, 6 or 12 (respectively 4, 3, 2 or 1 peak(s) per year). GMI data were square root transformed to obtain normality while the percentage of gonads in spent stage and the percentage of heterotrophic contribution to the lucinid diet were logit-transformed (Warton & Hui 2011). Dry mass measurements of 2 *Loripes* specimens collected in February 2010 were lost during processing. Each month, at every sampling occasion, individuals were collected from one or two sediment cores collected within an area of 1 m^2, and therefore, these individuals cannot be treated as independent. To account for this pseudoreplication, we treated month as a random effect.

Model selection was based on the Akaike information criterion corrected for small sample sizes (AIC_c; Burnham & Anderson 2002). ΔAIC_c values were used to compare the relative explanatory value of the models, with ΔAIC_c defined as the difference between the AIC_c value of the best-fitting model and each respective model in the set. AIC_c weights, which indicate the relative likelihood of a model given the data and set of models, were also calculated to provide a relative weight of evidence for each model (Burnham & Anderson 2002). The effect of a parameter was considered significant when adding this parameter to the model reduced the AIC_c with at least two points.

A Pearson correlation analysis was performed to evaluate the relationship between monthly mean values of environmental factors, feeding mode, and variables describing the body mass and reproductive cycle. In contrast to air temperature, precipitation and wind speed, sediment temperature data were lacking for some months during our study period. Given the significant relationship between sediment temperature and air temperature (t = 5.29, df = 7, P = 0.001), we only used air temperature, wind velocity and precipitation as environmental factors in this analysis.

All analyses were performed in R (R Development Core Team 2013, version 3.0.0). For non-linear mixed effects models, the R-package ‘nlme’ (Pinheiro *et al.* 2013) was used.

Results

Environmental variability

The mean monthly sediment temperature showed a clear seasonal trend with a minimum in January (19.5°C) and a maximum in September (29.1°C; Fig. 2.1A). At Nouadhibou, the mean monthly air temperature paralleled mean monthly sediment temperatures at the study site, ranging from 20.6°C in January to 26.5°C in September. The observed seasonal trend in sediment and air temperature was consistent between years, although mean monthly sediment and air temperature in January–May 2009 were on average 2°C lower than in January–May 2010 (Fig. 2.1A). We also observed a seasonal cycle in wind speed during our study period (Fig. 2.1B), with mean monthly wind speeds being relatively high in spring and early summer (March–July; mean speed (±SD) 7.6 ± 0.7 m s^{-1}) and relatively low in winter (November–February, mean speed (±SD) 4.8 ± 0.6 m s^{-1}). The observed

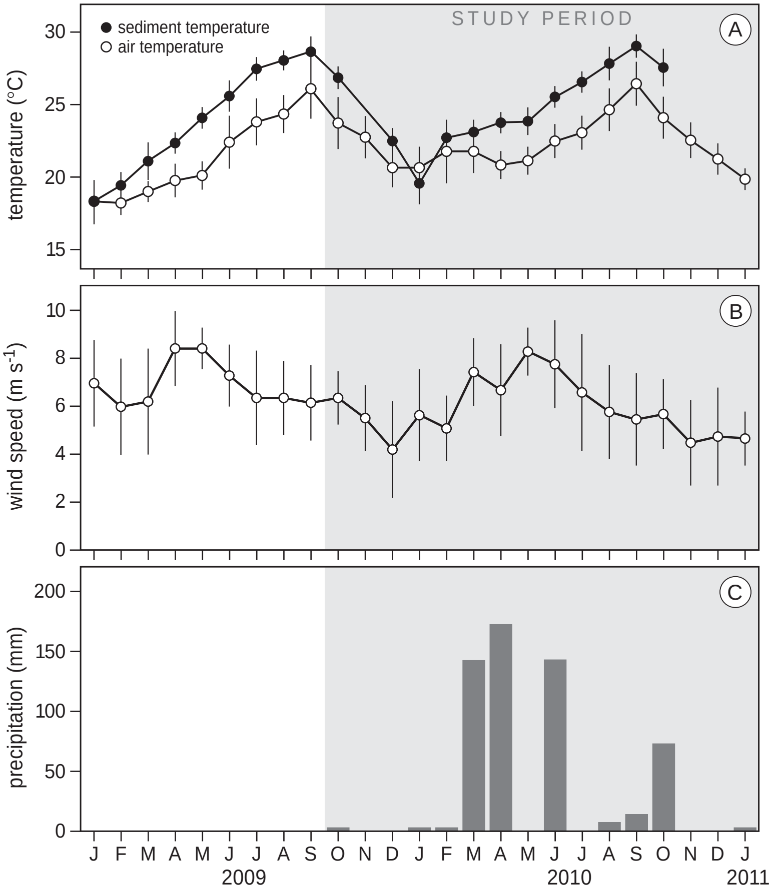

Figure 2.1 Environmental conditions at Banc d'Arguin during our study period (indicated by the period enclosed by the grey area) and in the nine months preceding our study period. (A) Mean monthly sediment temperature (˚C, mean ± SD, solid dots) and mean monthly air temperature (˚C, mean ± SD, open dots). (B) Mean monthly wind speeds (m s^{-1}, ± SD). (C) Total monthly precipitation (mm).

seasonal trend in wind speed was consistent between years, although mean annual wind speed in 2009 was with 6.5 m s^{-1} on average 0.4 m s^{-1} higher than in 2010 (Fig. 2.1B). During the study period, total monthly precipitation (mm) at Nouadhibou was mainly restricted to March, April and June with a small peak in September–October, while there was no rainfall in the 9 months preceding our study period (Fig. 2.1C).

Body mass and reproduction

A plot of the proportion of sexed animals against shell height indicated that sexual differentiation for both males and females started at a minimum shell height of ~4 mm (see Appendix I, Fig I.1). For individuals with a shell height ≥ 7 mm almost all specimens (86%) could

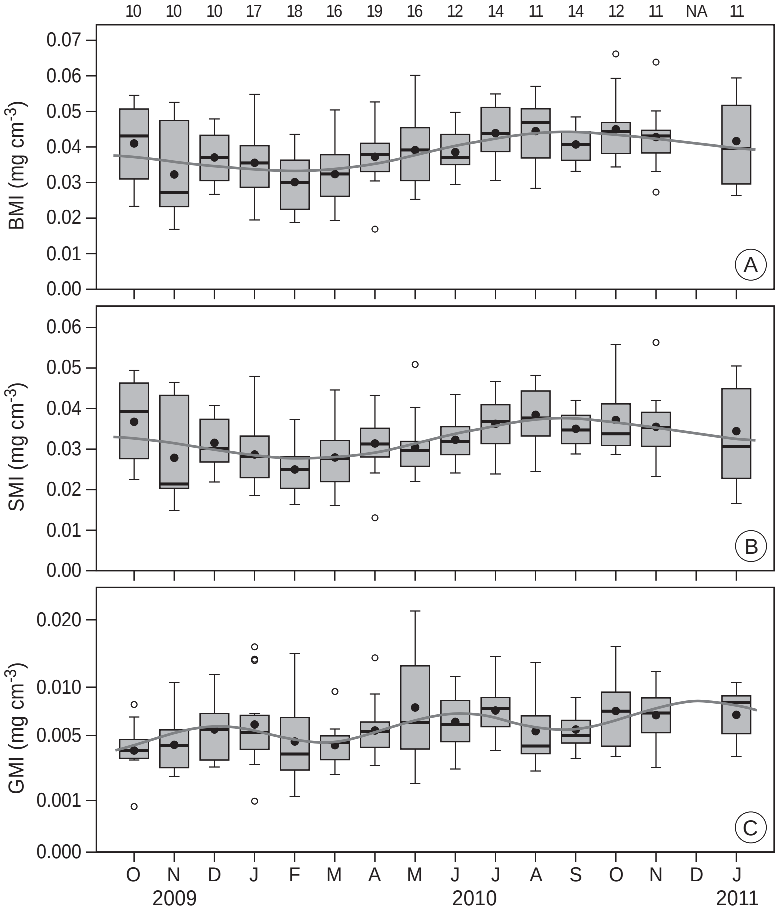

Figure 2.2 (A) Body mass index (BMI) (mg cm^{-3}), (B) somatic mass index (SMI) (mg cm^{-3}), and (C) gonadal mass index (GMI) over time for *L. lucinalis* with shell height ≥ 7 mm. Curves represent the best supported model ($\beta_0 + \beta_1 \cdot$ Month + Season) predictions. Box-and-whisker plots give the mean (filled dot), median (horizontal line inside the box), interquartile range (box), range (bars), and outliers (open dots). Numerals above Box-and-whisker plots indicate samples sizes.

be sexed, therefore we only used specimens ≥ 7 mm in shell height ($N = 203$ individuals) for further analyses.

Mean monthly Body Mass Index (BMI) and Somatic Mass Index (SMI) reached high values in late summer (i.e., July–September) and low values in winter (i.e., January–March) while both BMI and SMI increased over time (Fig. 2.2A, B). For both BMI and SMI the full model that fitted one peak per year ($\beta_4 = 12$) was best supported (Table 2.1). Gonadal Mass Index (GMI) also increased with time, but showed different seasonal patterns (Fig. 2.2C), with relatively high values in winter (December–January) and early summer (May–July) and relatively low values in early spring (February–March) and late summer

Table 2.1 Non-linear mixed effect model results evaluating the best fit for β_4 for body, somatic and gonadal mass indices and percentage of heterotrophic contribution to the diet of *L. lucinalis*, and non-linear model results evaluating the best fit for β_4 for proportion of gonads in spent stage. Season was described as the function $\beta_2 \cdot \sin(2\pi \cdot (\text{Month}-\beta_3)/\beta_4)$. Models are listed in order of ΔAIC_c (AIC_c: Akaike's information criterion corrected for small sample size). Results in bold indicate best supported model.

	β_4	No. of model parameters	ΔAIC_c	Akaike weight
Body mass index				
$\beta_0+\beta_1\cdot$Month+Season	12	6	0.00*	0.97
$\beta_0+\beta_1\cdot$Month	-	4	7.83	0.02
$\beta_0+\beta_1\cdot$Month+Season	3	6	9.57	0.01
$\beta_0+\beta_1\cdot$Month+Season	6	6	10.64	0.00
$\beta_0+\beta_1\cdot$Month+Season	4	6	11.96	0.00
* AIC_c = –1316.78				
Somatic mass index				
$\beta_0+\beta_1\cdot$Month+Season	12	6	0.00*	0.99
$\beta_0+\beta_1\cdot$Month	-	4	10.82	0.00
$\beta_0+\beta_1\cdot$Month+Season	3	6	13.04	0.00
$\beta_0+\beta_1\cdot$Month+Season	6	6	14.37	0.00
$\beta_0+\beta_1\cdot$Month+Season	4	6	14.78	0.00
* AIC_c = –1361.95				
Gonadal mass index (square root transformed)				
$\beta_0+\beta_1\cdot$Month+Season	6	6	0.00*	0.87
$\beta_0+\beta_1\cdot$Month	-	4	4.76	0.08
$\beta_0+\beta_1\cdot$Month+Season	3	6	7.34	0.02
$\beta_0+\beta_1\cdot$Month+Season	4	6	8.13	0.01
$\beta_0+\beta_1\cdot$Month+Season	12	6	8.14	0.01
* AIC_c = –1007.93				
Heterotrophic contribution (logit transformed)				
$\beta_0+\beta_1\cdot$Month+Season	12	6	0.00*	1.00
$\beta_0+\beta_1\cdot$Month	-	4	11.52	0.00
$\beta_0+\beta_1\cdot$Month+Season	3	6	14.54	0.00
$\beta_0+\beta_1\cdot$Month+Season	6	6	16.05	0.00
$\beta_0+\beta_1\cdot$Month+Season	4	6	16.10	0.00
* AIC_c = –29.31				
Proportion spent (logit transformed)				
$\beta_0+\beta_1\cdot$Month+Season	6	5	0.00*	0.99
$\beta_0+\beta_1\cdot$Month	-	3	9.94	0.01
$\beta_0+\beta_1\cdot$Month+Season	12	5	15.78	0.00
$\beta_0+\beta_1\cdot$Month+Season	4	5	17.27	0.00
$\beta_0+\beta_1\cdot$Month+Season	3	5	17.60	0.00
* AIC_c = 39.56				

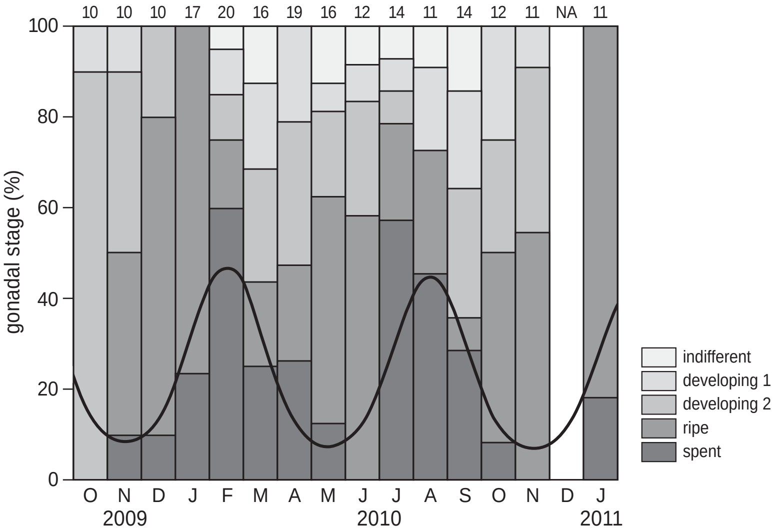

Figure 2.3 Gametogenic cycle of *L. lucinalis* based on gonadal stages from microscopic observations of smear samples of individuals ≥7 mm in shell height. Curves represent the best supported model ($\beta_0 + \beta_1 \cdot$Month + Season) predictions. Numerals above columns indicate samples sizes.

(August–September). For GMI, the full model that fitted two peaks per year ($\beta_4 = 6$) was best supported (Table 2.1). The fitted curve based on the optimal model prediction showed that GMI peaked in June and December, which corresponds to the months prior to spawning (Fig. 2.2C).

The distribution of gonad stages during our study period shows that *Loripes* has a semi-annual gametogenic cycle, with high gametogenic activity between March and July and between September and February (Fig. 2.3). Two major spawning periods, indicated by increasing microscopic observation of *spent* stages between January and February and between June and August are evident (Fig. 2.3). For the percentage of gonads in *spent* stage, the full model that fitted two peaks per year ($\beta_4 = 6$) was indeed best supported (Table 2. 1). The fitted curve based on this best supported model prediction showed that the percentage of gonads in *spent* stage peaked in February and August, which corresponds to the months after spawning (Fig. 2.3). Combining results of GMI and percentage of gonads in *spent* stage indicated two major spawning events, one in winter between January and February and a second one in summer between July and August.

Having determined the months in which the majority of the adult *Loripes* population is in the *spent* stage (Fig. 2.3), we could use all examined individuals in these breeding months (i.e., February and July) to investigate the size at first reproduction. (i.e., the smallest size-class where percentage of gonads in *spent* stage is higher than 50%) in the *Loripes* population. This resulted in a size at first reproduction of ~7.5 mm in shell height (see Appendix I, Fig. I.2).

Carbon stable isotopes

The δ^{13}C values presented in Table 2.2 show that carbon isotope signatures for non-gill and total body tissue of *Loripes* were closer to those of its bacterial endosymbionts, as corrected for fractionation than to the isotopic signatures typical of bivalves that suspension-feed on benthic POM (*Dosinia*).

The δ^{13}C values of both gill and non-gill tissues of *Loripes* collected between October 2009 and January 2011 were compared to determine isotopic differences (note that we have used the correction factor (+ 0.76‰) for δ^{13}C values of gill and non-gill tissues to account for the depletion effect of formalin). There was a significant relationship between gill δ^{13}C values and δ^{13}C values of non-gill tissue ($y = -7.52 + 0.65x$, $F_{1,46} = 100.5$, $P < 0.001$), with δ^{13}C values of gill tissue being on average 1.0 ± 0.4 ‰ (mean ± SD) more depleted. The average mean monthly δ^{13}C value of gill tissue was -24.7‰, whereas the average mean monthly δ^{13}C value of non-gill tissue was -23.7‰

Relatively low δ^{13}C values for the non-gill tissues were observed in November 2009 and from February to June 2010 (Fig. 2.4). Our calculations show that on average 23% of the carbon nutrition is obtained by feeding on benthic POM, with a minimum mean monthly heterotrophic contribution observed in March 2010 (16%) and a maximum mean monthly heterotrophic contribution observed in September 2010 (31%). The full model that fitted one peak per year ($\beta_4 = 12$) was best supported (Table 2.1, Fig. 2.4).

Factors affecting nutrition and resource allocation

The Pearson correlation analysis showed significant correlations between nutrition and environmental and biological variables: a positive correlation exists between the percentage

Table 2.2 Carbon stable isotope ratios (‰) in *Loripes lucinalis*, an enriched bacterial fraction separated from intact gills of *L. lucinalis*, and a specialist suspension feeder (*Dosinia isocardia*) collected in May 2008 from Banc d'Arguin, Mauritania. All investigated individuals (*Loripes*, $N = 22$; *Dosinia*, $N = 2$) measured between 7 and 11 mm in shell height and were stored frozen (-20°C) prior to isotope analysis.

		δ^{13}C (‰)	average
Loripes lucinalis			
	Bacterial pellet[1]	-27.4	-27.2
		-27.0	
	Non-gill tissue[2]	-23.7	-23.6
		-23.5	
	All body tissue	-24.4	-24.3
		-24.2	
Dosinia isocardia			
	All body tissue	-15.6	-15.5
		-15.3	

[1] Data for 10 gill pairs.
[2] Data for non-gill tissue pooled for 10 specimens.

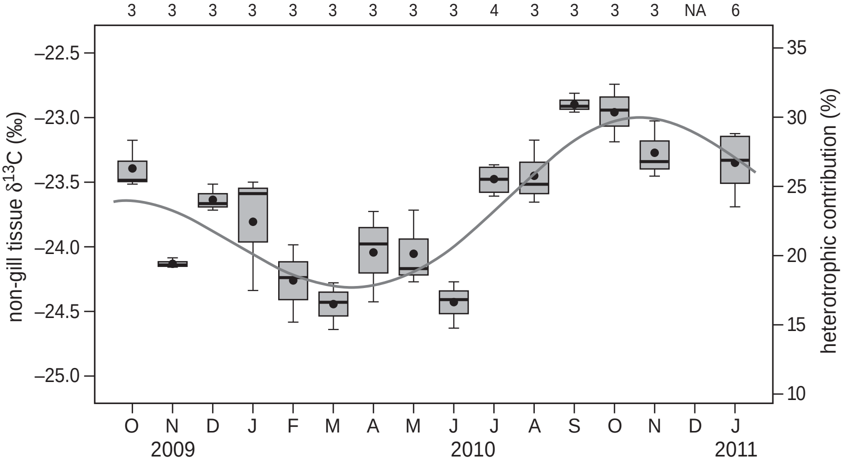

Figure 2.4 δ^{13}C values for non-gill tissues (enriched by 0.76‰ to correct for the depletion effect of tissue preservation in formalin), as well as the percentage of heterotrophic contribution to the carbon diet of *L. lucinalis* with shell height ≥7 mm over time. Curves represent the best supported model (β_0 + β_1 ·Month + Season) predictions.

of heterotrophic contribution to the diet of *Loripes* and both BMI and SMI, SMI and air temperature, BMI and SMI, BMI and GMI, and between precipitation and wind velocities (Fig. 2.5). In addition, wind speed was negatively correlated with heterotrophic contribution to the diet of *Loripes* (Fig. 2.5).

Discussion

The mean δ^{13}C value of -15.5‰ for small suspension-feeding *Dosinia* can be considered to reflect a dietary carbon dominated by resuspended microphytobenthos (Lebreton *et al.* 2012) and subsequently enriched by ~1‰ due to trophic interactions (Peterson & Fry 1987). The dissolved carbon fixed by sulphide-oxidizing bacteria is more δ^{13}C-depleted than that fixed by photosynthetically active organisms (Spiro *et al.* 1986; Ruby, Jannasch & Deuser 1987). Indeed, δ^{13}C values for *Loripes* are substantially more depleted in δ^{13}C than values of specialist suspension-feeding bivalves (e.g., *Dosinia*), thus indicating a significant carbon input from autotrophic endosymbiotic bacteria (on average 77%; Fig. 2.4).

The mean annual δ^{13}C value of -23.7‰ for non-gill tissues of *Loripes* measured in this study was within the range of those reported for *Codakia orbicularis*, a large lucinid bivalve living in seagrass beds in the tropical Caribbean (Berg & Alatalo 1984), while it was slightly more enriched (+1.7‰) than the δ^{13}C values obtained for mature *Loripes* from a sub-tropical Mediterranean *Z. noltii* seagrass bed (Rossi *et al.* 2013). The observed mean δ^{13}C of purified fractions of endosymbionts isolated from the gills of *Loripes* (δ^{13}C = -27.2‰) was also slightly more enriched than δ^{13}C values estimated by Rossi *et al.* (2013)

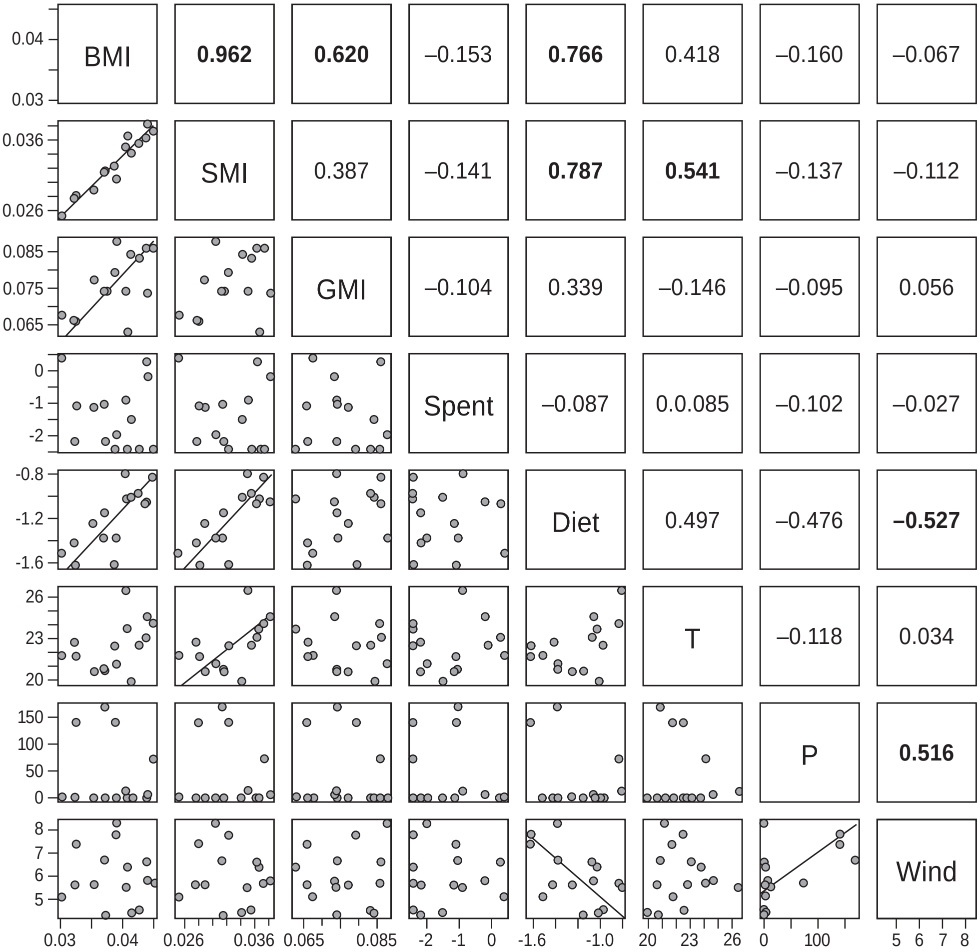

Figure 2.5 Matrix of scatterplots (lower panel) and Pearson's coefficients (upper panel) of correlations between body mass index (BMI, mg.cm^{-3}), somatic mass index (SMI, mg.cm^{-3}), gonadal mass index (GMI, mg.cm^{-3}), percentage of mature individuals classified as *spent* (Spent), percentage of heterotrophic contribution to the diet of mature individuals (Diet), air temperature (T, °C), precipitation (P, mm) and wind velocity (Wind, m s^{-1}). Monthly mean values ($N = 15$) were used for all variables. GMI data were square root transformed and data expressed in percentages were logit transformed to obtain normality. Significant correlations are indicated with a reduced major axis regression line through the data. Bold values represent significant correlations ($P < 0.05$).

for *Loripes* from a sub-tropical *Z. noltii* seagrass bed (ranging from -30 to -28‰). Temperature-dependent isotopic enrichment could play a role here, as experimental studies showed carbon isotopic enrichment with increasing temperature (Sackett *et al.* 1965; Power, Guiguer & Barton 2003).

When taking into account our measures of reproductive activity (i.e., GMI and percentage of gonads in *spent* stage), *Loripes* showed strong reproductive periodicity with gametogenesis being initiated in both March and September resulting in major spawning events during January–February and June–August (Fig. 2.3). This is supported by the model that fitted two peaks per year ($\beta_4 = 6$) being best supported for GMI and percentage of gonads in *spent* stage (Table 2.1). The latter suggests that some individuals may have spawned in June. However, in view of the observation that GMI increased from June to July and that the minimum GMI values in July were still relatively high indicating that spawning had only just commenced, we suggest that this spawning event was mainly restricted to July. That we predicted size at first reproduction to be reached at ~7.5 mm shell height, suggests that the choice to only include *Loripes* with shell heights ≥7 mm for analysis of seasonality of reproductive activity is justified.

The reproductive cycle shown here for a tropical area bears striking similarities to that of a temperate *Loripes* population studied by Johnson and Le Pennec (1994). Not only do both populations show semi-annual spawning, the duration of gametogenesis (i.e., ~4 months) is identical too. The only differences being the timing and intensity of the spawning events, with major spawning events in tropical *Loripes* between January–February and July–August compared to a major spawning event in May and a minor spawning event in November–December in temperate *Loripes* (Johnson & Le Pennec 1994). Given that the reproductive cycle of *Loripes* remains similar across a 30º latitude gap, we may reject our hypothesis that gametogenic development and breeding is more continuous in *Loripes* at lower latitudes. However, the selective forces governing the reproductive cycle are likely to be different between these two populations.

Gametogenesis and spawning is generally considered to be triggered by environmental cues (Sastry 1979). In this study no significant correlation was found between environmental variables (i.e., air temperature, precipitation and wind speed) and both GMI and gonads in *spent* stage (Fig. 2.5). This is in agreement with Johnson and Le Pennec (1994), who found that for a temperate *Loripes* population environmental triggers of spawning were not readily apparent. However, the onset of gametogenesis in September coincides with decreasing temperatures, while the January spawning peak coincides with the start of a temperature rise (see Figs. 2.1, 2.3). This may suggests that in contrast to a threshold temperature, an abrupt change in temperature may have triggered the autumn–winter gametogenesis and spawning event in tropical *Loripes*. Although periodicity in temperature may initiate gametogenesis, it occurs only if sufficient energy stores have been built up or if an adequate food supply is present (Newell *et al.* 1982; MacDonald & Thompson 1986; Santos *et al.* 2011).

In marine invertebrates, as in other animals, food is the most important factor controlling growth (Clarke 1987). If we assume that seasonality in body mass represents variability in food availability, the increase in BMI from February to August would suggest ample food availability, while the drop in BMI observed between September and February might reflect food limitation during this period (Fig. 2.2A). Given that GMI and the percentage of gonads in ripe stage increased from September to January (Figs. 2.2C & 2.3, respectively), this decrease in BMI from August to February is mainly the result of SMI decreasing in this period (Fig. 2.2B). This would suggest that in winter reproduction is based on stored resources (‘capital’ already invested in the soma), which is referred to as ‘capital breeding’ (Stearns 1992; Jønsson 1997). By producing offspring from stored resources, capital breeders decouple feeding and reproduction in contrast to reproduction based on concurrent food intake, which is termed ‘income breeding’ (Stearns 1992; Jønsson 1997). This strategy would enable *Loripes* to reproduce in January at the onset of the next feeding season, thereby presumably increasing offspring fitness. However, given the cost of carrying stores and the finite risk of mortality between resource acquisition and deployment to reproduction (Jønsson 1997), good feeding conditions between February and September may explain why *Loripes* adopted an income breeding strategy during this period.

We observed a significant increase in the relative heterotrophic contribution between July and January, with a maximum of 31% in September (Fig. 2.4). Among the factors that could have caused such seasonality in organic composition (Kennicutt *et al.* 1992), sediment temperature increased from January to September, while $\delta^{13}C$ values of *Loripes* only started to become more enriched by July, which suggests that environmental temperature cannot explain the observed seasonality in $\delta^{13}C$ values of *Loripes* non-gill tissues. Although not significant, there was some support for a positive correlation between air temperature and heterotrophic feeding (Fig. 2.5). It remains difficult to assess whether the potential positive effect of increasing temperatures on microphytobenthos productivity and biomass as observed by de Jonge *et al.* (2012), may have caused an increase in heterotrophic feeding, or whether increasing temperature by itself caused $\delta^{13}C$ values of *Loripes* to be more enriched as described above.

A weakness of this study is our assumption that $\delta^{13}C$ values of bacterial endosymbionts, microphytobenthos and benthic suspension-feeding bivalves are constant over time. To our knowledge there are no studies that have investigated whether $\delta^{13}C$ values of chemosymbiotic bacterial endosymbionts vary seasonally. However, when investigating seasonal differences in $\delta^{13}C$ values of microphytobenthos in a temperate intertidal *Z. noltii* bed, Lebreton *et al.* (2012) found $\delta^{13}C$ values to be ranging between -13.8‰ in spring and -15.5‰ in winter, with a yearly average (± SD) of 14.5‰ (±1.1). In contrast, our $\delta^{13}C$ values of *Loripes* were more depleted in spring (Fig. 2.4), which suggests that the estimated degree of seasonality in heterotrophic diet could be too conservative.

Being a benthic-diatom-dominated trophic system, with low productivity and biomass of marine phytoplankton (Sevrin-Reyssac 1984; Honkoop *et al.* 2008), we assumed that the importance of marine phytoplankton in the diet of suspension-feeding bivalves was restricted. Compared to benthic microalgae with $\delta^{13}C$ values ranging between -18 to -11.5‰ (Lebreton *et al.* 2011 and references therein), $\delta^{13}C$ values of pelagic microalgae in estuaries are generally more depleted, ranging from -18 to -24 ‰ (Gearing *et al.* 1984). Given that plankton blooms occur in spring and to a lesser extent in autumn, the obtained average $\delta^{13}C$ value of -15.5‰ for benthic suspension-feeding *Dosinia* in May 2008 (Table 2.2) suggests that even in spring marine phytoplankton is not an important food source for small benthic suspension-feeding bivalves inhabiting the seagrass beds of Banc d’Arguin.

A positive correlation was found between heterotrophic feeding and both BMI and SMI (Fig. 2.5), suggesting that resuspended microphytobenthos may be of higher nutritional value compared to chemosynthetically produced food provided by endosymbiotic bacteria. This is also supported by the observed inter-annual differences in BMI, SMI and GMI, as in autumn 2010 when heterotrophic contribution was relatively high compared to the same period in 2009 (Fig. 2.4), BMI, SMI and GMI were also relatively high (Fig. 2.2). A flexible feeding mode may thus help chemosymbiotic bivalves to survive in environments where the concentration of sulphide, oxygen and particulate food may vary seasonally.

One of the primary factors that regulate microphytobenthos production and biomass is light availability and to a lesser extent temperature (de Jonge *et al.* 2012). Although we have not measured microphytobenthos production and biomass at Banc d'Arguin, it is striking to see that the seasonal variability in wind speeds described by Dedah (1993) and observed in this study, with relatively calm weather from September to January and relatively strong winds during spring and early summer (April to July; Fig. 2B) correlates negatively with the observed microphytobenthic carbon contribution to the diet of *Loripes* (Fig. 2.5). This may suggest that high wind speeds in spring and early summer increased water turbidity and decreased subsequent microphytobenthic productivity due to light limitation, ultimately causing a decrease in the microphytobenthic carbon contribution to the diet of *Loripes*. This would imply that tidal currents together with the relatively low wind speeds measured in winter were still sufficient to make benthic diatoms become suspended in the water column, thereby making them available to benthic suspension feeders.

However, there may be an alternative explanation. In marine sediments sulphide is mainly produced by anaerobic decomposition of organic matter by sulphate-reducing bacteria (Jørgensen & Fenchel 1974). These sulphate-reducing bacteria have a temperature regulation of their metabolic rate, resulting in higher bacterial sulphate reduction activity levels when temperature rises (Jørgensen 1977). Indeed, sediment sulphide concentration measured at the 8–12 cm sediment layer at our study site followed seasonal changes in temperature with relatively low sulphide concentrations measured in the coldest month of the year (January 2011), reaching 119 ± 69 (mean ± SE) µmol L^{-1} (L. L. Govers and J. de Fouw, *unpublished data*), and relatively high sulphide concentrations reaching almost 300 µmol L^{-1} in a relatively warm month (October 2010) (van Gils *et al.* 2012). When in contact with oxygen, hydrogen sulphide immediately reacts to form sulfur dioxide and water. Given the relatively high sulphide concentrations in October, we therefore assume that low pore-water oxygen concentrations are limiting carbon metabolite production by the sulphide-oxidizing gill-bacteria of *Loripes* during this period. This would be in agreement with Childress and Girguis (2011) who suggested that the high oxygen demand of chemoautotrophic or methanotrophic symbionts is perhaps the most limiting flux for the symbioses. Furthermore, oxygen limitation in the warm autumn months is also supported by the fact that the solubility of oxygen in seawater is negatively correlated with temperature.

Overall, we suggest that the high sediment temperatures in autumn caused lower pore-water oxygen concentration, which in turn limits the nutritional contribution of the sulphide-oxidizing endosymbiotic gill-bacteria to its lucinid host, for which the hosts tries to compensate by incorporation of supplementary heterotrophic nutrition in the form of resuspended benthic microalgae. The productivity of benthic microalgae may have been enhanced in this period as a result of increased light conditions due to decreased wind-driven turbidity. However, given the drop in BMI from September onwards, the supplementary incorporation of suspended POM to the diet of *Loripes* in this period appears to be insufficient for body mass maintenance. As suggested by van Gils *et al.* (2012), competition for benthic POM with more specialized suspension-feeding bivalves like *Dosinia* may have limited benthic POM-feeding by *Loripes*.

Regardless of its absolute contribution, this study shows that heterotrophic feeding has the potential to play a key role in influencing resource allocation strategies and the synchronization and timing of reproduction in *Loripes*, which may have important fitness consequences in terms of reproductive output, mating efficiency and offspring fitness and subsequent population dynamics. Since seagrass ecosystems are currently declining at increasing rates worldwide, leading to loss of biodiversity (Waycott *et al.* 2009), knowledge about the biological and environmental processes that regulate heterotrophic feeding behaviour and population dynamics of lucinid bivalves may aid seagrass management and conservation, as lucinid bivalves contribute to the detoxification of sediments (van der Heide *et al.* 2012).

Acknowledgements

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Appendix I

Section I.1

To characterize the habitat at our study site, we took four replicate 8-cm deep sediment cores (diameter 2 cm) on 7 October 2010 for analysis of median grain size (MGS) and percentage of silt/clay (particles < 63 µm). Our study site can be characterized by sediment with MGS of 51.4 µm (range 43.0–56.3) and with a percentage of silt/clay of 62.5% (V/V; range 57.2–72.7). The reported sediment characteristics at our study site are well within the range of earlier observed values for seagrass-covered intertidal sediments at Banc d'Arguin (Honkoop *et al.* 2008).

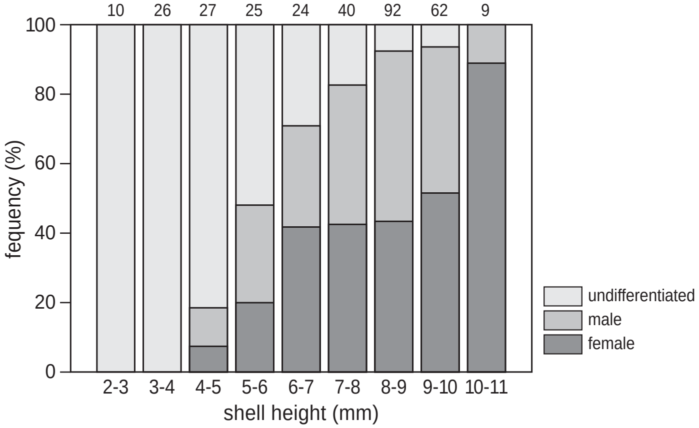

Figure I.1 Percentage of females, males and undifferentiated *L. lucinalis* per size-class (mm). Numerals above columns indicate samples sizes.

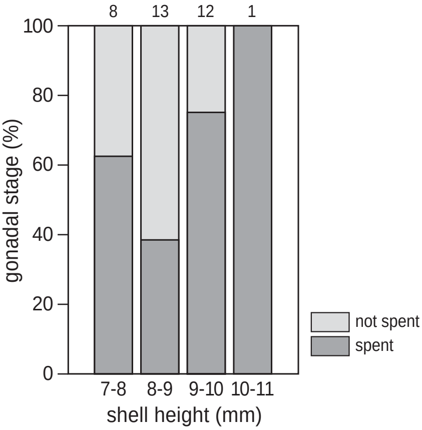

Figure I.2 Percentage of gonads in spent stage per size-class (mm) for mature *L. lucinalis* collected in February and July 2010 (i.e., the months in which the two major spawning events occurred). Numerals above columns indicate samples sizes.

Chapter 3

A three-stage symbiosis forms the foundation of seagrass ecosystems

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Abstract Seagrasses evolved from terrestrial plants into marine foundation species around 100 million years ago. Their ecological success, however, remains a mystery as natural organic matter accumulation within the beds should result in toxic sediment sulfide levels. Using a meta-analysis, a field study, and a laboratory experiment, we reveal how an ancient three-stage symbiosis between seagrass, lucinid bivalves and their sulfide-oxidizing gill-bacteria reduces sulfide stress for seagrasses. We found that the bivalve – sulfide-oxidizer symbiosis reduced sulfide levels and enhanced seagrass production as measured in biomass. In turn, the bivalves and their endosymbionts profit from organic matter accumulation and radial oxygen release from the seagrass roots. These findings elucidate the long-term success of seagrasses in warm waters and offer new prospects for seagrass ecosystem conservation.

Seagrass meadows are important ecological and thus economic components of coastal zones worldwide (Larkum, Orth & Duarte 2006; Waycott *et al.* 2009). In many areas, coral reefs and seagrass meadows are tightly linked habitats that form the basis for marine biodiversity (Nagelkerken 2009). Seagrasses serve as keystone habitat for migrating coral reef species as well as thousands of other animals, including waterbirds, fish, dugongs, manatees, and turtles; are important carbon and nutrient sinks; and are important to fisheries and coastline protection (Larkum, Orth & Duarte 2006; Nagelkerken 2009; Waycott *et al.* 2009). Dense seagrass meadows attenuate currents and waves and trap pelagic and benthic organic matter in the sediment (Larkum, Orth & Duarte 2006; van der Heide *et al.* 2007; van der Heide *et al.* 2011). Owing to a lack of oxygen in many coastal marine sediments, an important fraction of organic matter is decomposed by bacteria that use the abundant sulfate in seawater as an electron acceptor instead of oxygen and produce toxic sulfide as a metabolic end product (Jørgensen 1982). Although seagrasses transport oxygen into their roots and the surrounding rhizosphere (radial oxygen release) (Larkum, Orth & Duarte 2006; Calleja, Marba & Duarte 2007), sulfide production outpaces oxygen release under warmer conditions, resulting in sulfide accumulation and seagrass mortality (Larkum, Orth & Duarte 2006; Calleja, Marba & Duarte 2007; Koch *et al.* 2007). Seagrass beds tend to accumulate organic matter and so it is expected that seagrass beds would build up toxic sulfides and hence have a limited productivity and diversity (Larkum, Orth & Duarte 2006). But this is not the observed case and the underlying reason for the long-term persistence of seagrass ecosystems is an enigma (see Appendix II, Fig. II.1A).

We tested the hypothesis that a three-stage symbiosis between seagrasses, associated burrowing lucinid bivalves, and their symbiotic gill-bacteria contribute to reducing the cyclic build-up of sulfide (see Appendix II, Fig. II.1B to D). Paleo records suggest that the Lucinidae and their endosymbiotic relation date back to the Silurian (Liljedahl 1991; Distel 1998; Taylor & Glover 2000), but that they increasingly diversified since the evolutionary emergence of seagrasses in the late Cretaceous (Stanley 1977; Larkum, Orth & Duarte 2006; Taylor *et al.* 2011). Seagrass communities later became widespread in the Eocene and lucinid remains frequently occur in association with their deposits since (Taylor *et al.* 2011; Vermeij 2011). Lucinids and their gill-inhabiting bacteria have a symbiosis in which the bivalves transport sulfide and oxygen to their gills (see Appendix II, Fig. II.1D), where the bacteria oxidize sulfide for synthesizing sugars that fuel growth of both organisms (Cavanaugh 1983; Johnson, Diouris & Le Pennec 1994; Anderson 1995; Reynolds, Berg & Zieman 2007; Childress & Girguis 2011). We hypothesized that seagrass meadows may provide an optimal habitat for these bivalves and their symbionts by indirectly stimulating sulfide production by high organic matter input and by providing oxygen through radial oxygen release from the roots. In turn, lucinids remove sulfide, which could relieve any stress caused to seagrass growth by sulfide accumulation as organic matter is degraded (see Appendix, Fig. II.1A and B).

Indirect support for our hypothesis was provided by a worldwide meta-analysis of 84 studies describing the fauna of seagrass beds in 83 sites covering the entire climatic distribution of seagrasses, combined with a 110-point field survey that we conducted at Banc

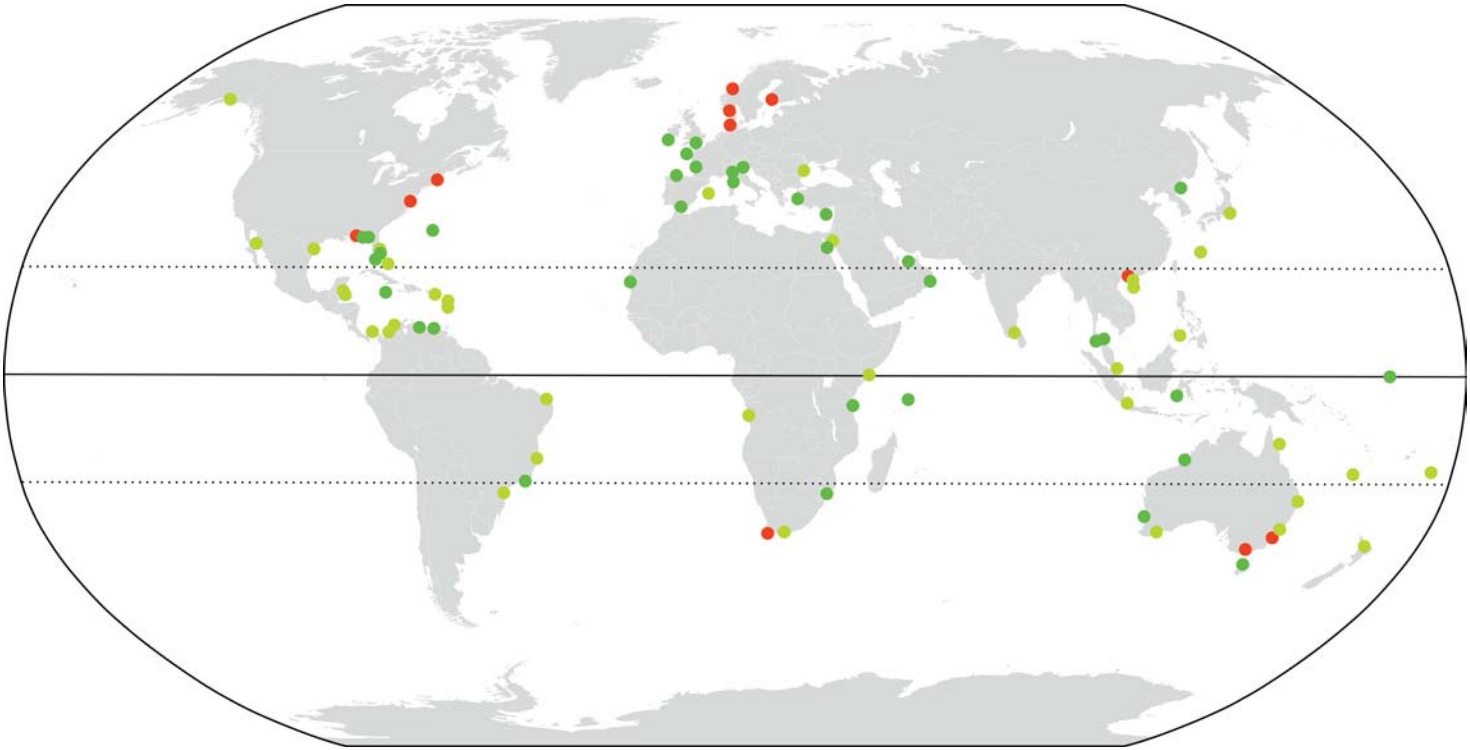

Figure 3.1 Presence (green; dark points are quantitative, light points are qualitative) and absence (red) of lucinids in seagrass ecosystems based on our meta-analysis. The bivalves were present in 97% (93% of the quantitative sites) of all tropical seagrass beds, 90% (83% of the quantitative sites) of the subtropical beds, and 56% (50% of the quantitative sites) of the temperate seagrass meadows. The seagrass-lucinid association spans six out of seven continents, at least 18 genera of lucinids, and 11 out of 12 seagrass genera (and *Ruppia* spp.). Only meadows of *Phyllospadix* spp., a seagrass genus that grows on bare rock, did not contain Lucinidae. The analyzed ecosystems generally contained high (~100 individuals per square meter) to extremely high densities (>1000 individuals per square meter) of lucinids (see Appendix, Table II.1).

d'Arguin, Mauritania (see Appendix II, Section II.1 *Materials and methods*). The meta-analysis reveals a relationship that covers 11 out of 12 seagrass genera (and *Ruppia* spp.) and at least 18 genera of Lucinidae (Fig. 3.1; Appendix II, Table II.1). Only meadows of *Phyllospadix* spp., a seagrass genus that grows on bare rock, do not associate with Lucinidae. The association spans six out of seven continents, with bivalve densities ranging from 10 to over 1000 individuals per square meter. The bivalves were present in 97% of the tropical seagrass sites, 90% of the subtropical meadows and 56% of the temperate seagrass beds surveyed, indicating that the association may be dependent on temperature-related sulfide production (Koch *et al.* 2007). Furthermore, results from our field study showed a positive correlation between seagrasses and lucinids that explained 42% of their respective variation (Pearson's correlation coefficient (r) = 0.65; Appendix II, Fig. II.2).

To experimentally test our hypothesis (see Appendix II, Fig. II.1B), we investigated the effects of sulfide oxidation by the lucinid bivalve *Loripes lacteus* on the production of the seagrass species *Zostera noltii* and the potential reciprocal benefits for *Loripes* in a full factorial experiment under controlled conditions (see Appendix, Section II.1). We set up *Zostera, Loripes*, *Zostera-Loripes* and bare sediment treatments in the top sections of 40 two-compartment columns (see Appendix II, Fig. II.3), which were placed in a large seawater basin. The lower compartment of each column contained anaerobic seawater and an injection tube through which sulfide was added twice a week in half of the columns. The injected sulfide was allowed to diffuse into the top section through a porous membrane.

The presence of *Loripes*, and to a lesser extent of *Zostera*, decreased sediment sulfide levels. After 5 weeks, pore water sulfide concentrations in the top sections of the sediment controls reached about 400 µM, whereas the semiweekly addition of sulfide caused levels to increase to nearly 2700 µM (Fig. 3.2A). The presence of *Zostera* decreased sulfide levels to ~200 µM in the controls and 2200 µM in the sulfide addition treatments. In contrast, sulfide levels remained low when *Loripes* was present (~15 µM), even in the sulfide addition treatments. As expected, the oxygen detection depth was reduced when sulfide was added, but increased when only *Loripes*, but not *Zostera*, was present because of sulfide-oxidation and intake of surface water (Fig. 3.2B). *Zostera* alone did not significantly affect sediment oxygen conditions. The joint presence of *Zostera* and *Loripes* enhanced oxygen detection depth beyond that of their separate effects.

Our experiment showed that *Zostera* production is facilitated by *Loripes*, both in the control and in the sulfide addition treatments. In the treatments without *Loripes*, sulfide addition reduced *Zostera* shoot biomass to 50% of the controls (Fig. 3.3A). Reduced shoot biomass was accompanied by decreased root biomass (Fig. 3.3B) and impaired phosphate uptake (see Appendix, section II.1). In contrast, the addition of *Loripes* increased *Zostera* shoot biomass 1.9-fold and root weight 1.5-fold, as seen in the sulfide-addition treatments. In the treatments without additional sulfide, the presence of *Loripes* increased both shoot and root weight by 1.4-fold and 1.3-fold, respectively.

Loripes condition, expressed as the flesh/shell dry weight ratio, was positively affected by sulfide addition (Fig. 3.3C). Furthermore, the addition of *Zostera* did not affect *Loripes* in the units where no sulfide was added but improved the bivalve's condition in the sulfide

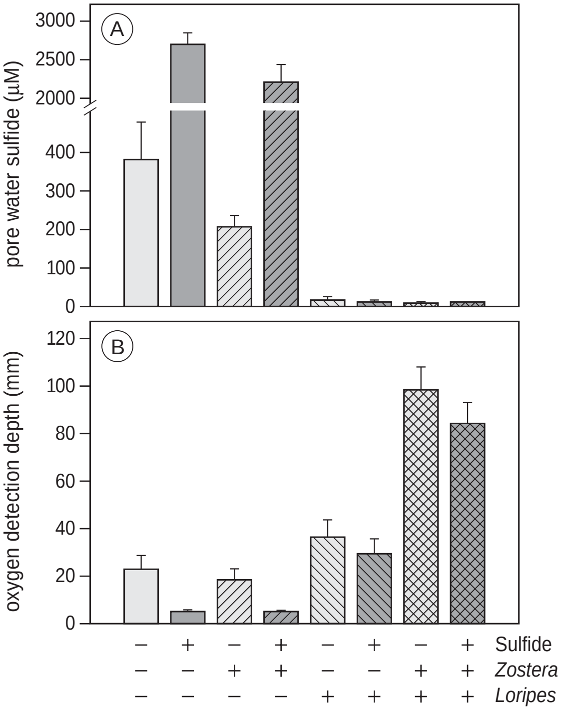

Figure 3.2 (A) Pore water sulfide concentrations and (B) oxygen detection depth after 5 weeks; error bars represent SEM ($N = 5$). Oxygen detection depth decreased as sulfide was added (analysis of variance (ANOVA): $F_{1,32} = 8.9$, $P < 0.006$). The presence of *Loripes* reduced sulfide levels (repeated measures ANOVA: $F_{1,32} = 268.8$, $P < 0.001$) and increased oxygen detection depth ($F_{1,32} = 125.0$, $P < 0.001$). Reduction of the sulfide concentration by *Zostera* alone was less, but still significant ($F_{1,32} = 6.8$, $P = 0.014$). That interactions occurred between *Zostera* and *Loripes* was apparent in the oxygen measurements ($F_{1,32} = 48.3$, $P < 0.001$) but was also significant in the sulfide data ($F_{1,32} = 7.8$, $P = 0.009$). The interaction between *Loripes* and sulfide was significant for the sulfide measurements ($F_{1,32} = 102.7$, $P < 0.001$) but not for the oxygen data ($F_{1,32} = 0.3$, $P = 0.578$).

treatments. As hypothesized, the positive effect of *Zostera* on *Loripes* seems to result from radial oxygen release from the seagrass roots (see Appendix II, Fig. II.1B). Although sulfide was almost completely removed in all *Loripes* treatments (Fig. 3.2A), the bivalve was less able to profit from the addition of sulfide in the absence of *Zostera* (Fig. 3.3C). This indicates that at least in the *Loripes* units without seagrass, sulfide was not completely oxidized by the symbiotic bacteria because of oxygen limitation.

Overall, our results confirm our hypothesis that a three-stage symbiosis between seagrass, lucinids and sulfide-oxidizing bacteria reduces sulfide stress in seagrass meadows. Even though radial oxygen release by *Zostera noltii* and of seagrasses in general is limited (Caffrey & Kemp 1991; Sand-Jensen *et al.* 2005), *Loripes* in our experiment clearly benefitted from the increased oxygen input in the sediment. In the field, the positive effects of seagrasses on lucinids are not confined to sediment oxygenation alone but also by indirectly

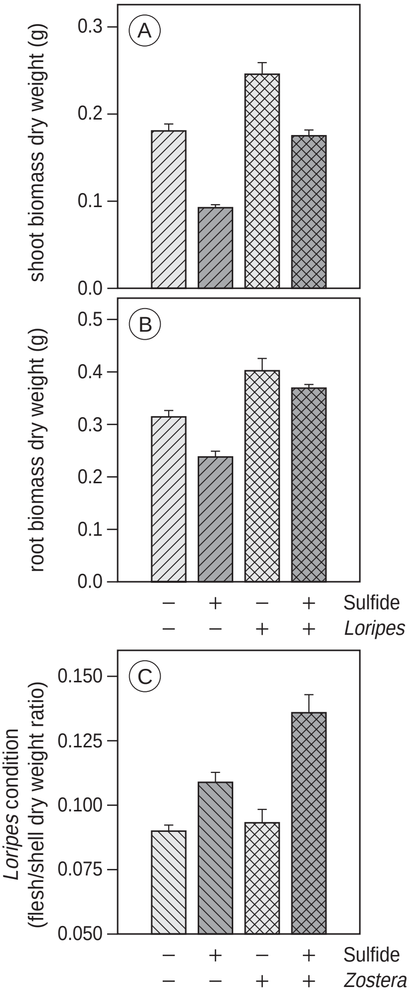

Figure 3.3 (A) *Zostera* shoot and (B) root dry weight biomass per column and (C) *Loripes* condition expressed as the dry weight flesh/shell ratio after 5 weeks; error bars represent SEM ($N = 5$). *Zostera* biomass was reduced by sulfide addition (ANOVA: shoots $F_{1,16} = 72.6$, $P < 0.001$; roots $F_{1,16} = 12.0$, $P = 0.003$), whereas the presence of *Loripes* had a positive effect on both shoot ($F_{1,16} = 61.3$, $P < 0.001$) and root biomass ($F_{1,16} = 50.2$, $P < 0.001$). We found no significant effects on rhizome biomass. *Loripes* condition was positively affected by both sulfide addition (ANOVA: $F_{1,16} = 37.3$, $P < 0.001$) and *Zostera* presence ($F_{1,16} = 9.0$, $P = 0.008$). We also found a significant positive combined effect of the presence of *Zostera* and sulfide on *Loripes* condition ($F_{1,16} = 5.4$, $P = 0.034$).

stimulating sulfide production and releasing dissolved organic molecules (Larkum, Orth & Duarte 2006; Reynolds, Berg & Zieman 2007). The positive effects of *Loripes* on *Zostera* in our experiment could not be explained by differences in nutrient availability (see Appendix, Section II.1). Plants were not nutrient-limited, but both *Zostera* and *Loripes* significantly lowered dissolved ammonium and phosphorus in the sediment pore water, whereas sulfide addition increased nutrient availability (see Appendix II, Fig. II.4). We found that in our experiment, the negative effects of sulfide addition on *Zostera* biomass could not fully be prevented by *Loripes* addition (Fig. 3.3A), despite the removal of almost all sulfide by *Loripes* after three days. As the observed experimental effects could not be attributed to differences in nutrient availability, this is most likely caused by the pulsed nature of our sulfide supply. This may have led to short periods of exposure of *Zostera* to toxic sulfide levels.

Coastal ecosystems, and seagrass meadows in particular, are currently declining at an alarming and increasing rate worldwide, leading to loss of biodiversity (Waycott *et al.* 2009). Extensive restoration efforts have had little success so far (<30%), despite their extremely high costs (± $ 100,000 per hectare) (Fonseca *et al.* 2002). Similar to the function of mycorrhizae, pollinators, or seed dispersers in terrestrial systems (van der Heijden *et al.* 1998; Bascompte & Jordano 2007; Bastolla *et al.* 2009), our findings indicate that restoration efforts should not only focus on environmental stressors like eutrophication, sediment run-off, or high salinity as a cause of decline but should also consider internal ecological interactions, such as the presence and vigor of symbiotic or mutualistic relations. Breakdown of symbiotic interactions can affect ecosystem functioning, with bleaching events in coral reefs as a clear example (Carpenter *et al.* 2008). Similar to the well-known symbiosis between corals and their unicellular algal endosymbionts (Baker 2003), we conclude that symbioses, rather than one defining species, forms the foundation of seagrass ecosystems.

Acknowledgements

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Appendix II

Section II.1 Materials and methods

Meta-analysis

To test the seagrass-lucinid association, we performed an extensive, worldwide meta-analysis that covered the entire climatic distribution of seagrasses. Criteria for including a study were: (1) seagrasses were present at the site, and (2) when Lucinidae were present, they were found inside the seagrass bed. In total, we analyzed 84 studies that sampled the fauna of seagrass beds in a total of 83 areas (temperature range = 1 to 33ºC, mean = 22ºC). Overall, 36 sites were from tropical areas, 31 from subtropical and 16 from temperate areas; quantitative data were available for 46 out of 83 sites. Apart from the geographical location of each site, and the seagrass and lucinid families found, we also report the annual seawater temperature range. These were obtained from freely available satellite imagery of the long-term monthly means (1971–2000) of the sea surface temperature (NOAA/OAR/ESRL/PSD, 2011).

Field study

We conducted a field survey at Banc d'Arguin (Mauritania) to test the strength of the relation between seagrass biomass and lucinid density. Banc d'Arguin consists of about 500 km^2 of intertidal flat dominated by mixed meadows of *Zostera noltii*, *Halodule wrightii* and *Cymodocea nodosa* that are inhabited by the lucinid bivalve *Loripes lacteus* (Wolff *et al.* 1993*a*). In total, we sampled 110 stations across seven intertidal flats. *Loripes* was sampled up to a depth of 20 cm using a cylindrical 15-cm diameter PVC core sampler and seagrass was sampled with a 7-cm diameter corer. Each sample was sieved over a 1-mm mesh sieve. Next, *Loripes* was counted and seagrass biomass was determined after drying for 24-h at 70ºC. Prior to linear regression analysis, *Loripes* counts and seagrass dry weight from the cores were transformed with the Box-Cox procedure to achieve normality and homoscedasticity (Box & Cox 1964).

Laboratory experiment

Organisms and sediment for the experiment were collected in Arcachon Bay (southwest France) and transported at 15ºC to the laboratory, where both species were separately acclimatized for three weeks in 100-L polyethylene tanks. *Zostera* units contained 15 cm of sediment and 20 cm of surface water; *Loripes* tanks contained 30 cm of sediment and 5 cm of surface water. We used artificial seawater (33-35 PSU Tropic Marin at 20ºC) throughout the acclimatization period and during the experiment; pH was kept at 8.1 to 8.3 by CO_2 aeration. Light period was 16 h day^{-1}; intensity at the leaf surface was 300 $\mu mol\ m^{-2}\ s^{-1}$, similar to growing season conditions in the field (Isaksen & Finster 1996). During this three-week period, we did not observe any bivalve mortality, and seagrasses exhibited healthy vegetative growth.

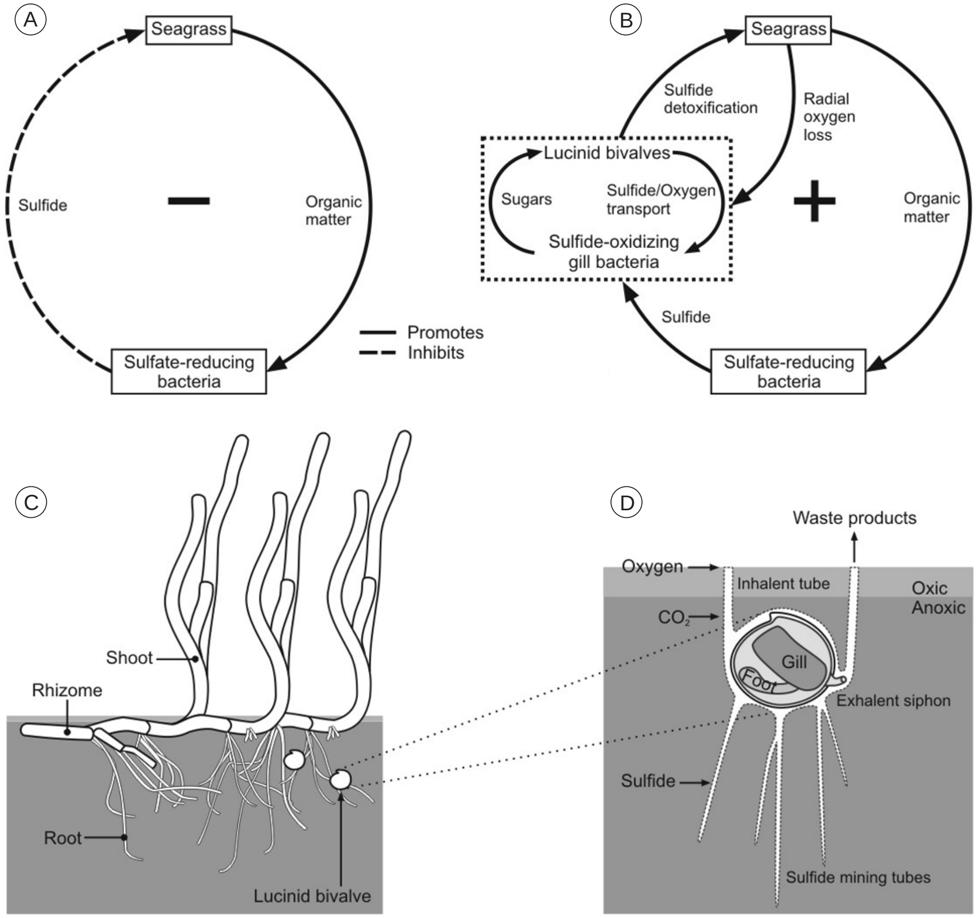

Figure II.1 (A) Seagrasses generally create a negative feedback on their own growth through organic matter accumulation, which stimulates production of toxic sulfide by heterotrophic sulfate-reducing bacteria. (B) We propose in this study that the presence of lucinid bivalves and their sulfide-oxidizing gill-symbionts breaks the negative feedback, resulting in a network of positive interactions. (C) The bivalves are found in high abundances in the root zones of seagrass meadows in warmer, mild temperate to tropical regions where sulfide production rates are high. (D) They occur in the anoxic zone of the sediment and use their highly extensile foot to create tubes for sulfide mining, export of waste products and import of oxygen and CO$_2$ from the sediment pore water and surface water (Anderson 1995; Reynolds, Berg & Zieman 2007). Both sulfide and oxygen are transported to the gills where chemoautotrophic bacteria oxidize sulfide for synthesizing sugars that fuel growth of both the bacteria and the bivalve (Johnson, Diouris & Le Pennec 1994; Anderson 1995; Reynolds, Berg & Zieman 2007; Childress & Girguis 2011).

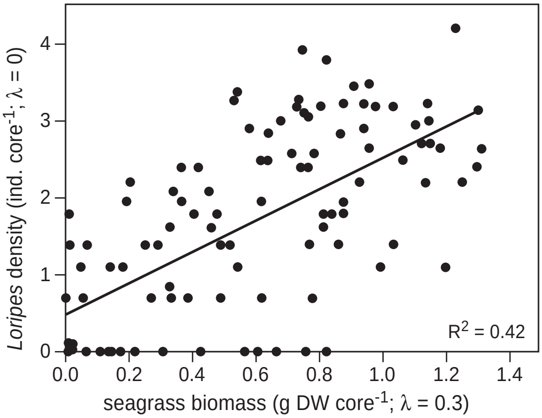

Figure II.2 Positive correlation (Pearson's $r = 0.65$) between seagrass biomass and *Loripes* density on Banc d'Arguin. *Loripes* counts and seagrass dry weight from the cores were transformed using the Box-Cox procedure prior to plotting and the regression analysis (see Appendix, Section II.1 *Materials and methods*).

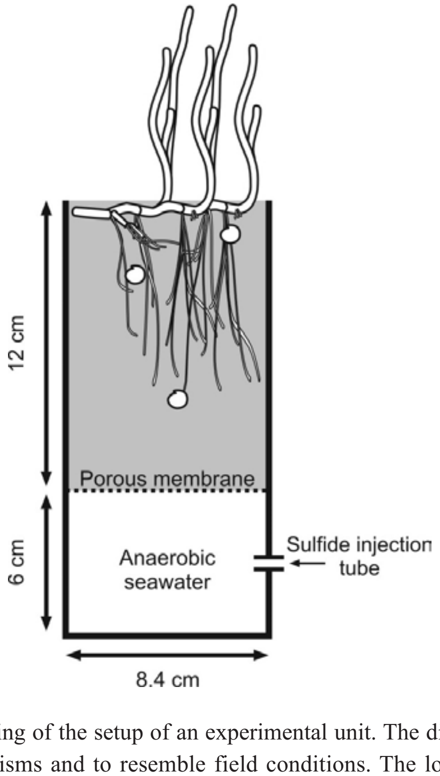

Figure II.3 Schematic drawing of the setup of an experimental unit. The dimensions of the top section were chosen to fit the organisms and to resemble field conditions. The lower section was kept large enough to allow rapid mixing and upward diffusion. Sulfide was injected twice a week in the sulfide addition treatments and allowed to diffuse from the lower compartment into the upper section through a 0.1 mm porous membrane.

Experimental setup

The lower 6 cm tall sections of 40 two-compartment PVC columns (diameter 8.4 cm) were filled with anaerobic seawater (see Appendix, Fig. II.3). These 330-ml sections contained an injection tube and were separated from their upper compartments through a porous 0.1 mm membrane. Sediment was passed through a 1 mm sieve and transferred to the upper 12 cm tall sections (surface area: 0.0055 m^2). Depending on the treatment, each unit then received either 1) *Loripes*, 2) *Zostera*, 3) both *Zostera* and *Loripes*, or 4) no further treatment. Nine *Loripes* specimens were added to each *Loripes* treatment (~1600 ind. m^{-2}; mean shell length ~9 mm) and 5 seagrass ramets with 2 or 3 shoots (12 shoots in total) were planted in each unit containing *Zostera* (~2200 sh. m^{-2}; ~0.12 g shoot, ~0.06 g rhizome and ~0.03 g DW root biomass per column). Each ramet contained one apical shoot to allow vegetative growth. Pilot experiments showed that this approach ensured consistent colonization of the units within the two-week adjustment period, with no detectable mortality of the plants. Densities of both species were well within reported ranges of densities in the field (up to 23000 shoots m^{-2} for *Zostera* and 3700 ind. m^{-2} for *Loripes*) (Vermaat & Verhagen 1996; Johnson, Fernandez & Pergent 2002; van der Geest *et al.* 2011).

A full factorial experiment was designed with eight treatments and five replicates per treatment. The columns were randomly placed in a 40-cm high 250-L polyethylene basin where water flow and oxygen saturation (measured with a 556 Multi Parameter Sampler, Yellow Springs Instruments) were maintained by two aquarium water pumps, and pH was kept constant (8.1–8.3) by CO_2 aeration. After setup, the units were allowed to adjust for two weeks. During this period, sulfide levels in the treatments containing *Loripes* stabilized at ~7 µM, while sulfide in treatments without *Loripes* increased to ~233 µM. Following the adjustment period, the experiment was performed for five weeks. Sulfide levels in the lower compartments of the sulfide addition treatments were increased twice a week by 3.3-ml injections of 100 mM Na_2S solution with pH adjusted to sediment conditions (pH 7.5) with HCl, while control treatments were injected with anaerobic water. Before each injection, we used 5 cm Rhizon samplers to extract 3 ml of pore water from the main root zone (top 6 cm) of each upper compartment into vacuumized 30 ml flasks containing 3 ml Sulfide Anti-Oxidation Buffer (SAOB). After each sampling, columns were re-randomized in the basin to minimize possible differences in light levels and water flow velocities between units. Sulfide concentrations were determined immediately with an ion selective silver/sulfide electrode (Thermo Scientific (USA), Orion 9416 BN; reference electrode: Orion 900200). Oxygen detection depth was measured after five weeks with an oxygen-sensitive microelectrode (Microscale Measurements, 1-mm tip). Ammonium, nitrate and total dissolved phosphorus in the sediment pore water were also measured after five weeks. We used 5 cm Rhizon samplers to extract 10 ml of pore water from the main root zone (top 6 cm) of each upper compartment into vacuumized 30 ml flasks. Ammonium and nitrate concentrations were determined colorimetrically. Ammonium was measured with salicylate (Lamers, Tomassen & Roelofs 1998) and nitrate was determined by sulfanilamide after reduction of nitrate to nitrite in a cadmium column (Wood, Armstrong & Richards 1967). Dissolved phosphorus was measured on an Inductively Coupled Plasma emission spectrophotometer

(ICP; Spectroflame, Spectro). Total nitrogen concentration in *Zostera* leaves was measured in freeze-dried tissues by a CNS analyzer (type NA1500; Carlo Erba Instruments, Milan, Italy) (Lamers, Tomassen & Roelofs 1998). Total phosphorus was measured by ICP after digestion with nitric acid (Lamers, Tomassen & Roelofs 1998). *Zostera* shoot, root and rhizome biomass and *Loripes* flesh were measured as dry weight after 24 h of freeze-drying. *Loripes* shell weight was measured after drying for 24 h at 70ºC. *Loripes* condition was expressed as flesh/shell dry weight ratio, which is a commonly used size-and-age independent measure of fitness in bivalves (Lucas & Beninger 1985). Sulfur contents in the *Loripes* tissues were measured on ICP, following nitric acid digestion.

STATISTICAL ANALYSES

Data were tested for normality prior to analysis. Sulfide data were analyzed with Repeated-Measures three-factor ANOVA. All other variables were analyzed by two- or three-factor ANOVA. All relevant and/or significant effects and interactions are mentioned in the figure legends or in the Appendix, Section II.2. A complete overview of the statistical output for Figs. 3.2, 3.3 and Appendix-Fig. II.4 is provided in the Appendix, Table II.2.

Section II.2

Both *Zostera* and *Loripes* significantly lowered dissolved ammonium and phosphorus in the sediment pore water, while sulfide addition increased their availability (see Appendix, Fig. II.4). Nitrate concentrations were 0.8 ± 0.9 µM (mean ± SD) on average with no significant differences between treatments. Mean leaf nitrogen and phosphorus content were 1.78 ± 0.26 and 0.15 ± 0.02% dry weight, respectively, which is around reported median values from the field for both (1.8 and 0.2% DW, respectively) (Duarte 1990). None of the treatments had any significant effect on leaf nitrogen. Leaf phosphorus content was unaffected by *Loripes*, but decreased significantly in the sulfide addition and sulfide addition with *Loripes* treatments (from 0.17 ± 0.01 to 0.13 ± 0.01% DW; ANOVA: $F_{1,16} = 29.0$, $P < 0.001$). Apparently, high sulfide levels impaired phosphorus uptake by *Zostera* in the

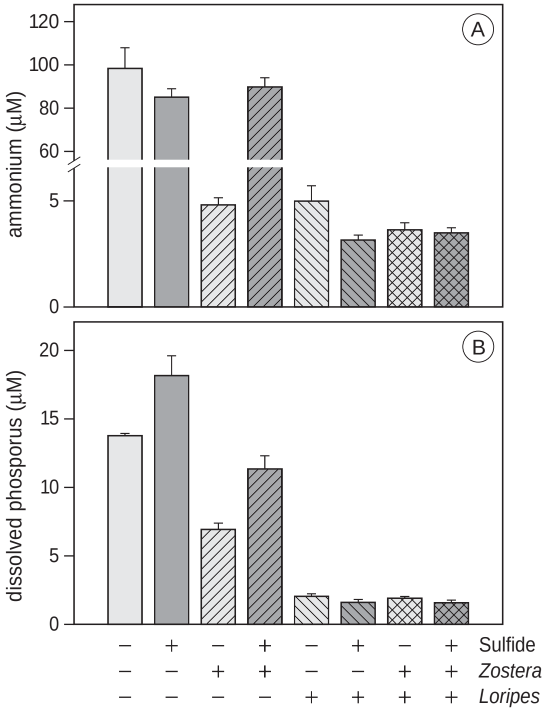

Figure II.4 Pore water ammonium and dissolved phosphorus contents after five weeks; error bars represent SEM ($N = 5$). Ammonium (A) was lowered significantly by *Zostera* (ANOVA: $F_{1,32} = 59.7$, $P < 0.001$) and *Loripes* ($F_{1,32} = 505.9$, $P < 0.001$), while sulfide addition caused an increase ($F_{1,32} = 35.2$, $P < 0.001$). We found significant interactions between all treatments (Z*L: $F_{1,32} = 57.1$, $P < 0.001$; Z*S: $F_{1,32} = 73.3$, $P < 0.001$; L*S: $F_{1,32} = 39.3$, $P < 0.001$; Z*L*S: $F_{1,32} = 68.5$, $P < 0.001$). The treatment effects on dissolved phosphorus (B) were similar to ammonium, with significant effects of *Zostera* ($F_{1,32} = 58.2$, $P < 0.001$), *Loripes* ($F_{1,32} = 562.1$, $P < 0.001$) and sulfide addition ($F_{1,32} = 19.6$, $P < 0.001$). We found significant interactions of *Zostera* and *Loripes* ($F_{1,32} = 55.1$, $P < 0.001$), and *Loripes* and sulfide addition ($F_{1,32} = 28.2$, $P < 0.001$).

sulfide addition treatment, leading to decreased leaf phosphorus content, despite high dissolved phosphorus availability in the pore water (see Appendix, Fig. II.4). Our pulsed sulfide addition also seemed to impair phosphorus uptake in the sulfide addition with *Loripes* treatment, which, by interacting with the reduced dissolved phosphorus pool, may have limited growth of *Zostera* under our conditions (Fig. 3.3).

Sulfide addition resulted in a significant increase in the relative (ANOVA: $F_{1,16} = 13.8$, $P = 0.002$) and absolute sulfur content (ANOVA: $F_{1,16} = 24.1$, $P < 0.001$) in the flesh of the bivalves. Relative sulfur content was 2.0 ± 0.2 % (g:g) in the control treatments and 3.0 ± 0.9 % in the sulfide addition treatments. The total amount of sulfur stored in *Loripes* tissues per unit was 1.3 ± 0.2 mg in the control treatments and 3.0 ± 1.1 mg in the sulfide addition treatments. These results suggest that the increased sulfide availability led to increased storage of sulfur in the tissues of the bivalves, for instance as sulfur granules in the gills (Anderson 1995). We found no significant effects of *Zostera* on *Loripes* sulfur content.

Table II.1 Lucinid bivalve densities found in seagrass beds. These data provide a basic indication of the association between seagrasses and lucinids worldwide.

Area	Temp.	Clim.	Seagrass genus	Lucinid genus	Density	Source
North America						
Alaska	5 – 13	Temp.	*Zostera*	Lucinidae	p	Jewett *et al.* (1999); Dean & Jewett (2001)
Boston Harbor	3 – 18	Temp.	*Zostera*		0	Leschen, Kessler & Estrella (2009)
Chesapeake Bay	1 – 23	Temp.	*Zostera*		0	Orth (1973)
Apalachee Bay, Florida	18 – 29	Subtr.	*Syringodium*, *Thalassia*	*Codakia*	+	Lewis & Stoner (1981)
Biscayne Bay, Florida	24 – 30	Subtr.	*Halodule*, *Syringodium*, *Thalassia*	*Anodontia, Codakia, Lucina*	++/+++	Moore *et al.* (1968)
Florida Bay, Florida	24 – 30	Subtr.	*Halodule*, *Syringodium*, *Thalassia*	*Anodontia, Codakia, Lucinesca*	++/+++	Reynolds, Berg & Zieman (2007)
Indian River lag., Florida	23 – 29	Subtr.	*Thalassia*	*Lucina*	p	Mikkelsen, Mikkelsen & Karlen (1995)
St. Joseph's Bay, Florida	18 – 29	Subtr.	Thalassia	*Lucina*	++/+++	Fisher & Hand (1984)
Pensacola Bay, Florida	18 – 29	Subtr.	*Halodule*		0	Stoner *et al.* (1983)
Redfish Bay, Texas	19 – 29	Subtr.	*Halodule, Thalassia*	*Anodontia, Lucina, Phacoides*	p	Tunnell *et al.* (1996)
Gulf of California, Mexico	19 – 30	Subtr.	*Zostera, Halodule, Ruppia*	*Codakia, Divalinga*	p	Torra Cosio & Bourillón (2000)
Bahia de Chetumal, Mexico	27 – 29	Trop.	*Syringodium, Thalassia*	*Codakia, Lucina*	p	Quesada *et al.* (2004)
Turneffe Islands, Belize, Mexico	27 – 29	Trop.	*Thalassia*	*Codakia, Parvilucina*	p	Hauser, Oschmann & Gischler (2007)
Bocas del Toro, Panama	27 – 29	Trop.	*Halodule, Syringodium, Thalassia*	*Codakia, Diplodonta Lucina, Phacoides*	p	Continental Shelf Associates, Inc. (1995)
Bahama's	24 – 29	Trop.	*Thalassia*	*Codakia*	p	Brissac (2009)
Jamaica	27 – 29	Trop.	*Thalassia*	*Anodontia, Codakia, Ctena, Divaricella, Lucina, Parvilucina*	+++/++++	Greenway (1995); Jackson (1972)
St Croix, Virgin Islands	26 – 29	Trop.	*Halodule, Syringodium, Thalassia*	*Codakia, Divalinga, Lucina, Parvilucina*	p	Ferguson & Miller (2007)
Guadeloupe	26 – 29	Trop.	*Thalassia*	*Anodontia, Codakia*	p	Gros, Liberge & Felbeck (2003)
Martinique	26 – 29	Trop.	*Thalassia*	*Lucina*	p	Brissac (2009)
Bermuda	19 – 28	Subtr.	*Thalassia*	*Codakia, Ctena*	++/+++	Aurelia (1969); Schweimanns & Felbeck (1985)

Table II.1 Continued

Area	Temp.	Clim.	Seagrass genus	Lucinid genus	Density	Source
South America						
Bahia de Neguange, Colombia	26 – 29	Trop.	*Thalassia*, *Syringodium*	*Codakia*, *Lucina*, *Anodontia*	p	Diaz (2003)
Santiago de Tolú, Colombia	27 – 29	Trop.	*Thalassia*	*Lucina*	p	Otero-Otero & Lobo (2009)
Morrocoy, Venezuela	26 – 28	Trop.	*Thalassia*	*Codakia*	+	Bitter-Soto (1999)
Mochima Bay, Venezuela	25 – 28	Trop.	*Thalassia*	*Codakia*	+++	Díaz (2004)
Parracho de Maracajaú, Brazil	26 – 28	Trop.	*Halophila*, *Halodule*	*Codakia*, *Divaricella*	p	Martinez (2008)
Abrolhos Bank, Bahia Brazil	25 – 28	Trop.	*Halodule*, *Halophila*	*Codakia*, *Ctena*, *Parvilucina*	p	Dutra *et al.* (2005)
Ilha do Japonês, Brazil	23 – 27	Trop.	*Halodule*	*Codakia*, *Divaricella*	++++	Marques & Creed (2000); Creed & Kinupp (2011)
Ilha do Mel, Paranaguá, Brazil	18 – 26	Trop.	*Halodule*	*Lucina*	p	Couto & Savian (1998)
Europe						
Western Atlantic, Norway	6 – 13	Temp.	*Zostera*		0	Fredriksen *et al.* (2010)
Skagerrak, Atlantic, Norway	4 – 17	Temp.	*Zostera*		0	Fredriksen *et al.* (2010)
Baltic, Finlan	1 – 16	Temp.	*Zostera*		0	Bostrom & Bonsdorff (1997)
Sylt, Wadden Sea	4 – 18	Temp.	*Zostera*		0	Reise (1985)
South England	8 – 17	Temp.	*Zostera*	*Lucinoma*	+	Dando, Southward & Southward (1986)
South Ireland	9 – 17	Temp.	*Zostera*	*Lucinoma*	+++	Dale, McAllen & Whelan (2007)
Brittany, France	10 – 17	Temp.	*Zostera*	*Loripes*, *Lucinoma*, *Lucinella*	+++/++++	Hily & Bouteille (1999): Monnat (1970)
Arcachon, France	12 – 21	Temp.	*Zostera*	*Loripes*	++	Blanchet *et al.* (2004)
Eo estuary, Atlantic coast, Spain	13 – 19	Temp.	*Zostera*	*Loripes*	++/+++	De Paz *et al.* (2008)
Mediterranean, Spain	15 – 23	Subtr.	*Zostera*	*Lucinella*	+++	Rueda & Salas (2008)
Mallorca, Spain	14 – 25	Subtr.	*Posidonia*	*Ctena*, *Loripes*, *Lucinella*	p	Centeno (2008)
Corsica, France	13 – 24	Subtr.	*Cymodocea*	*Loripes*	+++/++++	Johnson, Fernandez & Pergent (2001)
Prelo Bay, Ligurian Sea	13 – 23	Subtr.	*Posidonia*	*Lucinella*	++/+++	Harriague, Bianchi & Albertelli (2006)

Table II.1 Continued

Area	Temp.	Clim.	Seagrass genus	Lucinid genus	Density	Source
Europe						
Venice lag., Italy	10 – 26	Subtr.	*Cymodocea*, *Zostera*	*Loripes*	+++/++++	Sfriso, Birkemeyer & Ghetti (2001); Pranovi *et al.* (2000)
Izmir Bay, Turkey	15 – 23	Subtr.	*Zostera*	*Loripes*	++	Cinar *et al.* (1998)
Cyprus	17 – 28	Subtr.	*Posidonia*	*Loripes*, *Myrtea*	+	Argyrou, Demetropoulos & Hadjichistophorou (1999)
Black Sea, Romania	6 – 24	Temp.	*Zostera*	*Loripes*, *Lucinella*	p	Nocolaev & Zaharia (2011)
Africa						
Banc d'Arguin, Mauritania	18 – 26	Subtr.	*Cymodocea*, *Halodule*, *Zostera*	*Loripes*	+++/++++	van der Geest *et al.* (2011)
Baia da Corimba, Angola	22 – 29	Trop.	*Halodule*	*Loripes*	p	Van-Dunem do Sacramento Neto dos Santos (2007)
Kismayo, Somalia	25 – 29	Trop.	*Halodule*, *Thalassia*	*Codakia*, *Lucina*	p	Chelazzi & Vannini (1980)
Zanzibar, Tanzania	25 – 29	Trop.	*Cymodocea*, *Thalassia*, *Enhalus*, *Thalassodendron*	Lucinidae	++/++++	Eklof *et al.* (2005)
Mahé, Seychelles	26 – 30	Trop.	*Thalassia*	*Anodontia*, *Codakia*, *Ctena*	++	Taylor & Lewis (1970)
Inhaca, Mozambique	23 – 27	Trop.	*Cymodocea*, *Halodule*, *Zostera*	*Anodontia*, *Cardiolucina*, *Loripes*, *Lucina*, *Pillucina*	++	De Boer & Prins (2002)
Langebaan lag., South-Africa	15 – 19	Subtr.	*Zostera*		0	Siebert & Branch (2005)
Swartvlei estuary, South-Africa	17– 22	Subtr.	*Zostera*	*Loripes*	p	Whitfield (1989)
Asia/Pacific						
Jordan, Red Sea	21 – 28	Subtr.	*Halodule*, *Halophila*	*Rasta*	p	Taylor *et al.* (2005)
Egypt, Red Sea	22 – 29	Subtr.	*Cymodocea*, *Halodule*, *Halophila*	*Cardiolucina*, *Divaricella*, *Pillucina*, *Wallucina*	++++	Zuschin & Hohenegger (1998)
United Arab Emirates	21 – 33	Subtr.	*Halodule*, *Halophila*	*Anodontia*, *Pillucina*	++++	Feulner & Hornby (2006)
Oman	25 – 28	Trop.	*Halodule*, *Halophila*	*Pillucina*	++++	This study
Palk Bay, India	27 – 30	Trop.	*Cymodocea*, *Halodule*, *Syringodium*, *Thalassodendron*	*Codakia*, *Lucina*	p	Gophinadha-Pillai & Appukuttan (1980)

Table II.1 Continued

Area	Temp.	Clim.	Seagrass genus	Lucinid genus	Density	Source
Asia/Pacific						
Posyet Bay, Sea of Japan	2 – 21	Temp.	*Zostera*	*Pillucina*	+++	Kharlamenko *et al.* (2001)
Tokyo, Bay of Japan	16 – 26	Subtr.	*Zostera*	Luncinidae	p	Whanpetch (2011)
Okinawa, Japan	22 – 29	Subtr.	*Cymodocea*, *Enhalus*, *Halodule*, *Halophila*, *Thalassia*	*Codakia*, *Epicodakia*	p	Yamaguchi (1999)
Guangxi, China	20 – 29	Trop.	*Halodule*, *Halophila*, *Zostera*		0	Huang (2008)
Guangdong, China	21 – 29	Trop.	*Halodule*, *Halophila*	*Pillucina*	p	Huang (2008)
Hainan, Chin	22 – 29	Trop.	*Cymodocea*, *Enhalus*, *Halodule*, *Thalassia*	*Pillucina*	p	Huang (2008)
Tubbataha Reefs, Philipines	27 – 30	Trop.	*Halodule*, *Halophila*, *Thalassia*	*Epicodakia*	p	Yamaguchi (1999)
Kungkrabaen Bay, Thailand	28 – 30	Trop.	*Halodule*	*Anodontia*, *Indoaustriella*, *Pillucina*	++++	Meyer *et al.* (2008)
Had Chao Mai, Thailand	28 – 30	Trop.	*Cymodocea*, *Enhalus*, *Halodule*, *Halophila*, *Thalassia*	*Pillucina*	++++	Nakaoka, Mukai & Chunhabundit (2002)
Pulau Semakau, Singapore	28 – 29	Trop.	*Cymodocea*, *Enhalus*, *Halodule*, *Halophila*, *Syringodium*, *Thalassia*	*Anodontia*	p	Tan & Yeo (2010)
Bone Batang, Indonesia	28 – 30	Trop.	*Cymodocea*, *Enhalus*, *Halodule*, *Halophila*, *Thalassia*	Lucinidae	+++	Vonk, Christianen & Stapel (2008)
Banten Bay, Indonesia	28 – 30	Trop.	*Cymodocea*, *Enhalus*, *Halodule*, *Halophila*, *Syringodium*, *Thalassia*	*Anodontia*, *Codakia*	p	Kuriandewa (2008)
Tongapatu, Tonga	23 – 27	Trop.	*Halodule*	*Codakia*, *Epicodakia*	p	Yamaguchi (1999)
Tarawa Atoll	28 – 29	Trop.	*Thalassia*	*Codakia*, *Wallucina*	++/+++	Paulay (2000)

Table II.1 Continued

Area	Temp.	Clim.	Seagrass genus	Lucinid genus	Density	Source
Oceania						
Roebuck Bay, Australia	25 – 30	Trop.	*Halodule*, *Halophila*	*Anodontia*, *Ctena*, *Divaricella*	+++	Piersma *et al.* (2006); this study
Lizard Island, Australia	25 – 29	Trop.	*Halophila*	*Anodontia*, *Chaviana*, *Wallucina*	p	Taylor & Glover (2008)
Moreton Bay, Australia	21 – 26	Subtr.	*Cymodocea*, *Halodule*,	*Anodontia*, *Pillucina* *Halophila*, *Zostera*	p	Taylor & Glover (2008)
Rottnest Island, Australia	19 – 23	Subtr.	*Posidonia*	*Wallucina*	+++/++++	Barnes & Hickman (1999)
South-West Australia	16 – 20	Subtr.	*Amphibolis*, *Posidonia*,	*Anodontia*	p	Hutchings, Wells & Kendrick (1991)
New South-Wales, Australia	19 – 24	Subtr.	*Halophila*	*Wallucina*	p	Gibbs, Maguire & Collett (1984)
New South-Wales, Australia	17 – 23	Subtr.	*Halophila*, *Zostera*		0	McKinnon *et al.* (2009)
Western Port, Victoria, Australia	13 – 18	Temp.	*Halophila*, *Zostera*		0	Edgar *et al.* (1994); Watson, Robertson & Littlejohn (1984)
Tasmania, Australia	12 – 16	Temp.	H*eterozostera*. *Ruppia*, *Zostera*	*Wallucina*	++/+++	Edgar, Barrett & Gradon (1999); Edgar, Barrett & Last (1999)
New Caledonia	24 – 28	Subtr.	*Cymodocea*, *Halodule*, *Thalassia*	*Anodontia*, *Codakia*, *Ctena*	p	Glover & Taylor (2007)
Slipper Island, New Zealand	15 – 21	Subtr.	*Zostera*	*Divaricella*	p	Schwarz, Morrison & Hawes (2006)

Temp. depicts the mean annual temperature range based on the sea surface temperature (ºC); Clim. indicates type of climate (tropical, subtropical or temperate); Lucinid density (spatial average): + = 1-10; ++ = 11-100; +++ = 101-1000; ++++ = >1000 ind. m^{-2} p = present (no abundance data); 0 = absent.

Table II.2 Overview of the statistical output from the analyses of the data presented in Figs. 3.2, 3.3, and Appendix-Fig. II.4.

Treatment	**df**	***F***	***P***
Sulfide measurements (Fig. 3.2A; repeated measures ANOVA)			
Zostera	1	6.8	0.014
Loripes	1	268.8	<0.001
Sulfide	1	109.7	<0.001
Zostera * Loripes	1	7.8	0.009
Zostera * Sulfide	1	2.2	0.150
Loripes * Sulfide	1	102.7	<0.001
Zostera * Loripes * Sulfide	1	2.4	0.127
Error	32		
Oxygen measurements (Fig. 3.2B; ANOVA)			
Zostera	1	39.3	<0.001
Loripes	1	125.0	<0.001
Sulfide	1	8.9	0.006
Zostera * Loripes	1	48.3	<0.001
Zostera * Sulfide	1	0.0	0.862
Loripes * Sulfide	1	0.3	0.578
Zostera * Loripes * Sulfide	1	0.5	0.505
Error	32		
Zostera shoot biomass (Fig. 3.3A; ANOVA)			
Loripes	1	61.3	<0.001
Sulfide	1	72.6	<0.001
Loripes * Sulfide	1	0.9	0.348
Error	16		
Zostera root biomass (Fig. 3.3B; ANOVA)			
Loripes	1	50.2	<0.001
Sulfide	1	12.0	0.003
Loripes * Sulfide	1	1.7	0.211
Error	16		
Loripes fitness (Fig. 3.3C; ANOVA)			
Sulfide	1	37.3	<0.001
Zostera	1	9.0	0.008
Sulfide * Zostera	1	5.4	0.034
Error	16		
Ammonium (Fig. II.4A; ANOVA)			
Zostera	1	59.7	<0.001
Loripes	1	505.9	<0.001
Sulfide	1	35.2	<0.001
Zostera * Loripes	1	57.1	<0.001
Zostera * Sulfide	1	73.3	<0.001
Loripes * Sulfide	1	39.3	<0.001
Zostera * Loripes * Sulfide	1	68.5	<0.001
Error	32		
Phosphorus (Fig. II.4B; ANOVA)			
Zostera	1	58.2	<0.001
Loripes	1	562.1	<0.001
Sulfide	1	19.6	<0.001
Zostera * Loripes	1	55.1	<0.001
Zostera * Sulfide	1	0.0	0.888
Loripes * Sulfide	1	28.2	0.000
Zostera * Loripes * Sulfide	1	0.0	0.965
Error	32		

Chapter 4

Suitability of calcein as an *in situ* growth marker in burrowing bivalves

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Abstract The fluorochrome calcein has been used in numerous growth studies of molluscs to internally mark calcified structures. Because of interspecific variations in marking success and possible effects on growth performance, methodological assessments of the suitability of calcein as a growth marker, especially in field contexts, remain necessary. Here we report on the effects of different calcein concentrations (100, 200, 400 and 800 mg l^{-1}) on fluorescent mark deposition, growth rate, density, body condition and size-frequency distribution of an intertidal infaunal bivalve species, *Loripes lacteus* (Linnaeus, 1758), using an outdoor immersion technique. To avoid stress caused by handling and transportation, *in situ* enclosures were placed at seagrass-covered patches during low tide, to which calcein solutions were added. After a 1.3- to 2.6-h period of exposure to calcein, the enclosures were removed. Sites were sampled three months later. All calcein concentrations produced live *L. lacteus* with a clear fluorescent shell mark, but the percentage successfully marked tended to increase with higher calcein concentrations. Furthermore, marking success and growth rate decreased significantly with shell size (i.e., age). Calcein concentration did not measurably affect shell growth rate, body condition and size-frequency distribution, but the numerical densities were lower for sites treated with calcein concentrations $\geq$400 mg l^{-1}. Our results suggest that *in situ* calcein-marking of burrowing lucinid bivalves with low concentrations (100–200 mg l^{-1}) is a non-invasive and rapid method to determine growth rate, provided that the lucinids are not too old.

Introduction

Growth rate is a key parameter in the context of an organism's life history, and therefore research efforts have concentrated on the development of suitable methods to measure growth. Because many bivalve species are commercially important and exploited (McLachlan *et al.* 1996), growth rates have been well studied in bivalves. Various methods were used to measure rates of increase in shell growth, including size-distribution analysis of single cohorts, analysis of size-increment following mark-and-recapture experiments using different labeling techniques, shell growth-ring analysis, internal growth lines analysis in shell sections, elemental analysis and analysis of stable oxygen isotopes (see Herrmann *et al.* 2009 and references therein). Size-frequency distribution analyses are widely used to estimate growth rate in temperate species with identifiable cohorts and peaked growth seasons. However, in (sub-) tropical species reproduction and growth often occur throughout the entire year, resulting in the absence of clearly defined cohorts (Sastry 1979; Urban 2001). In such species, mark-and-recapture experiments seem more suitable to estimate growth rates, the more so because they are inexpensive and easy to apply (Fujikura, Okoshi & Naganuma 2003). Yet, traditional marking methods (e.g., surface filing, edge notching, painting labels, tagging) involve the physical handling of the shells and removal from their natural habitat which may lead to disrupted shell growth (Jones, Thompson & Ambrose 1978). Furthermore, juveniles often cannot be marked due to their small size.

More recently, chemical techniques using fluorochromes (e.g., tetracycline, xylenol orange, alizarin red and calcein) have been used for growth studies on molluscs (Day, Williams & Hawkes 1995; Rowley & MacKinnon 1995; Kaehler & McQuaid 1999; Thébault *et al.* 2006; Riascos *et al.* 2007; Lucas *et al.* 2008; Herrmann *et al.* 2009). The fluorochromes are incorporated into newly mineralized calcium carbonate that makes up the shell, providing a bright green fluorescent reference growth mark that is visible under UV light from which subsequent shell growth can be measured (Wilson, Beckman & Dean 1987). Obviously, to be a suitable growth marker, fluorochromes should be non-toxic and should not adversely affect growth or survivorship. A number of laboratory studies on bivalve molluscs encouragingly indicate that the fluorochrome calcein provides long-lasting fluorescent shell marks without detectable negative effects on growth performance and survivorship (Eads & Layzer 2002; Heilmayer *et al.* 2005; Moran & Marko 2005; Riascos *et al.* 2007; Lucas *et al.* 2008). That these laboratory studies indicate calcein to be a safe growth marker does not necessarily mean that this will also be the case when used under field conditions (Kaehler & McQuaid 1999; Thébault *et al.* 2006; Herrmann *et al.* 2009), as the toxic effects of a chemical on an organism may differ between field and laboratory (McFarlane & Beamish 1987).

In the present study we examined the potential of the fluorochrome calcein as an *in situ* growth marker of the thin-shelled burrowing lucinid, *Loripes lacteus* (Mollusca; Bivalvia; Lucinidae), which lives on the seagrass-covered intertidal mudflats of Banc d'Arguin, Mauritania. We tested for the effects of calcein concentration on lucinid marking success, growth rate, survival, body condition and size-frequency distribution. As many other deeply burrowed bivalve species (Stanley 1970), *L. lacteus* is fragile and susceptible to stress by handling. The great advantage of this *in situ* marking method may be that physical stress is minimized because individuals are not touched or moved during the process. An effective non-invasive marker for fragile burrowing bivalves would be a welcome tool for measurements of secondary production in *L. lacteus* and other marine organisms with accretionary growing skeletons.

Materials and methods

Study site

The study was carried out at the intertidal flats close to Iwik, a small fishermen's village in the Banc d'Arguin, Mauritania (19° 52.42' N, 16° 18.50' W). The marking took place from 25 January to 1 February 2008 and the retrieval of marked bivalves between 24 April and 7 May 2008. The Banc d'Arguin is a major wintering site for migrating shorebirds and is also renowned as a nursery area for fish (Altenburg *et al.* 1983; Smit & Piersma 1989; Jager 1993). The tidal range is 1.5–2 m and the greater part of the intertidal flats is covered with dense seagrass beds, mainly *Zostera noltii* (Wolff & Smit 1990). Salinity varies between 38 and 42 ‰, but can reach values of more than 50 ‰ in sheltered creeks close to the shore (Wolff & Smit 1990). During the experiment, intertidal flat sediment temperature at 2 cm depth was measured every 15 minutes, with daily averages varying from 17.1˚C (27 January 2008) to 25.4˚C (28 April 2008; van der Geest *et al.*, *unpublished data*). In the seagrass-covered flats of our study area, *Loripes lacteus* is the most abundant bivalve species with an average density of 340–780 individuals per m^2 (Honkoop *et al.* 2008; pers. obs.) and maximum densities of 3700 individuals per m^2 (pers. obs. in April 2007). This lucinid bivalve species lives burrowed in the mud to maximum depths of 10 cm and its thin shells never grow larger than 16 mm (pers. obs.).

Calcein staining experiment

The experiment was conducted at seven tidal flats distributed around the Iwik peninsula (a 50 km^2 subsection of the Banc d'Arguin). Within each tidal flat we randomly selected a seagrass-covered site (coordinates of the seven sites were respectively 19°53.615' N, 16°19.619' W; 19°54.996' N, 16°18.881' W; 19°53.554' N, 16°18.822' W; 19°52.244' N, 16°18.210' W; 19°52.588' N, 16°17.260' W; 19°53.647' N, 16°16.561' W; 19°53.035' N, 16°16.370' W). To reach the study sites at low tide, and to work on the usually very muddy tidal flats, we always walked on snow rackets which prevented us from sinking in the mud and from locally damaging the nationally protected mudflats.

During low tide, at each study site, four 15 cm high PVC rings with a diameter of 30 cm were pushed 10 cm into the sediment (breaking through the seagrass-layer). The PVC rings were placed 2 m apart from each other, in a block design, to keep the environmental conditions at the four 'sub-sites' per site as similar as possible. Next, any overlaying water enclosed by the PVC rings was removed by gently scooping. To mark the bivalves, the area within each ring was immersed with a 500 ml calcein (Sigma, CAS 1461-15-0) solution of either 100, 200, 400 or 800 mg l^{-1} (Fig. 4.1A; solutions were made using 500-µm filtered ambient seawater). These concentrations were selected on the basis of previous studies (Day, Williams & Hawkes 1995; Kaehler & McQuaid 1999; Thébault *et al.* 2006). At each site, the four calcein concentrations were allocated randomly to each of the four PVC rings. The PVC rings were removed simultaneously per site, always just before being flushed by the incoming tide. As such, there were no differences in calcein immersion time between sub-sites within a site, but only between sites (2.1 ± 0.4 h (mean ± SD). Three months later, a sediment core (internal diameter 15.2 cm) was taken to a depth of 20 cm in the centre of each calcein-immersed sub-site and the content of each core was sieved through a 1-mm sieve and frozen for later analysis. During retrieval of the marked individuals, we also took a 10-cm deep sediment core (internal diameter 20 mm) at each site for analysis of median grain size (MGS) and percentage of silt/clay (particles < 63 µm). Additionally, a 10-cm deep seagrass core (internal diameter 70 mm) was taken in order to estimate seagrass abundance (expressed as g ash-free dry mass (AFDM) m^{-2}; for general methods see Honkoop *et al.* 2008). The seven sites can be characterized by sediment with MGS of 84.9 µm (range 28.0–160.7), with a percentage of silt/clay of 46.3% (V/V; range 14.2–82.5), a mean total seagrass biomass of 230.9 g AFDM m^{-2} (range 159.2–355.3), of which 82.4 g m^{-2} (range 18.2–162.7) was represented by the leaves and 148.6 g m^{-2} (range 86.4–214.0) by the roots (including rhizomes).

Sample preparation and detection of calcein marks

Frozen samples were transported to The Netherlands. Shell height was recorded for all individuals in each sample to the nearest 0.1 mm. From each individual, soft tissues were removed and transferred to porcelain crucibles, which were then dried for four days at 60°C. Dried tissues were weighed and incinerated (560°C) for 5 hours in order to determine AFDM (Honkoop 2003). Shells were cleaned in a H_2O_2-solution (35%) for 24 h to remove the periostracum, rinsed in tap water and dried at room temperature. Before further treatment, shells were stored in the dark to prevent any fluorescence decrease (Wilson, Beckman & Dean 1987). To investigate the presence of a calcein mark at the exterior of the shell, the valves of each shell were observed under an Olympus fluorescence microscope (SZX-12) exciting at 460–490 nm (SZX-RFL3 Cube). A marking quality index (MQI) was defined according to fluorescence intensity and length of the mark: 0 = no mark, 1 = poor mark, 2 = satisfactory mark, 3 = good mark.

Detection of absolute growth

In general, the *internal* calcein mark deposited in calcein-treated bivalves is used for growth estimation (Kaehler & McQuaid 1999; Thébault *et al.* 2006; Riascos *et al.* 2007; Herrmann *et al.* 2009). However, the problem with this procedure is that it is very time-consuming: before the internal calcein mark can be observed, the shell has to be sectioned and polished. Therefore, in order to speed up the measurements of growth estimation, we here aimed to determine growth on the basis of the calcein marks deposited at the exterior of the shell.

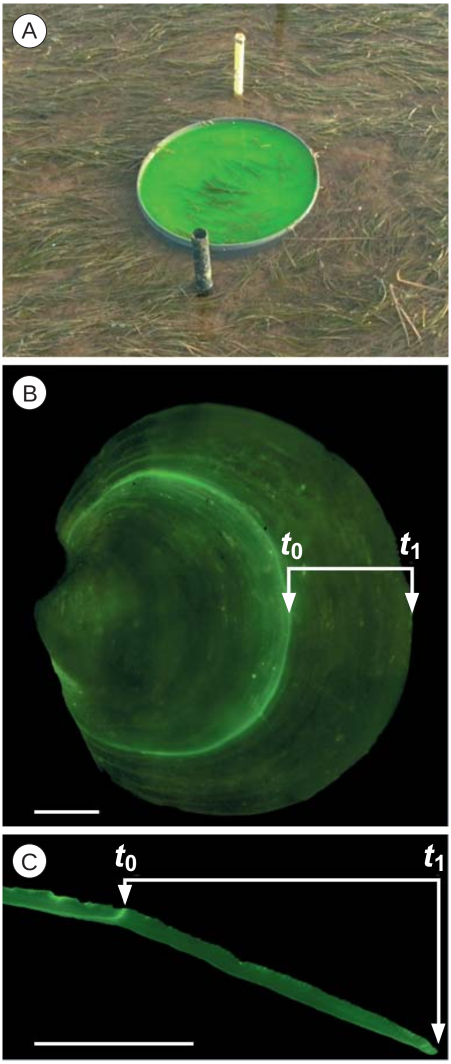

Figure 4.1 (A) *In situ* marking of burrowed bivalves by allocation of 500 ml calcein solution of 800 mg l^{-1} to a seagrass-covered sub-site at a particular site. (B) Photograph of right valve of a calcein marked *Loripes lacteus* shell, viewed with an I2/3 block filter (calcein concentration 800 mg l^{-1}). (C) Photomicrograph of sagittal section of the left valve of the same calcein marked shell. The position of growing edge at time of calcein administration is given by t_0; the position of growing edge 3 months later is given by t_1. Thus, t_0 to t_1 represents shell growth (increase in height). Scale bars: 1 mm.

By using a micrometer which was installed in the fluorescence binocular, we determined growth in those individuals that displayed a satisfactory or good calcein mark at the exterior of their shell (MQI scores >1, N = 261). Growth rate was expressed as the maximum growth axis between the fluorescent calcein mark at the exterior of the shell and the ventral margin to the nearest 0.01 mm divided by the interval (in days) between calcein administration at t_0 and collection of the shell at t_1 (Fig. 4.1B). Shell height at calcein marking (initial shell height) was expressed as the difference between the final shell height and the shell increase since marking.

In order to validate the assumption that the deposition of the calcein mark at the exterior of the shell can be used to determine growth, we randomly selected 20 marked (MQI >1) *L. lacteus* individuals of which we also estimated growth by using the internally deposited calcein mark (Fig. 4.1C). The selected valves were embedded in epoxy resin and after hardening for three days, the valves were sectioned sagittally along the maximum growth axis by using a diamond impregnated blade on an Isomet slow-speed saw machine. The cross-sections were polished on glass slides with different grades of Buehler silicium carbide powder (600-12-5-3 µm), and finally with 0.1 µm Buehler aluminum oxide suspension. The shell slides were observed under a Leitz DM RBE fluorescence microscope (Leica Mikroskopie & Systeme GmbH, Wetzlar, Germany) equipped with an Osram 50-W high-pressure Hg lamp and an I2/3 filter block (excitation filter BP450–490, dichroic mirror RKP510 and emission filter LP515). Growth was determined with a micrometer to the nearest 0.01 mm by measuring the distance between the internal calcein mark at t_0 and the ventral margin at t_1 (Fig. 4.1C).

A paired *t*-test revealed no significant differences in growth when either using the external or the internal calcein mark ($t = -0.240$, $df = 18$, $P = 0.81$). Therefore, growth data obtained by using the exterior calcein mark are used for further analysis.

Statistical analysis

Linear mixed modeling techniques (Pinheiro & Bates 2000; West, Welch & Galecki 2006; Zuur *et al.* 2009) were used to assess the effect of the calcein concentration on respectively quality of shell marking (MQI), growth (µm day^{-1}), density (in m^{-2}, a net measure of local dispersal, mortality and settlement in the intervening three months), final shell height (a net measure of size-selective mortality and/or migration) and body condition (AFDM corrected for shell height). When applicable, the R^2 value was calculated from the linear mixed model output by using a log-likelihood ratio test (Magee 1990). Due to non-normality of the MQI-data, we used mean MQI per sub-site. Previous studies have shown that marking quality can be related to shell size (Day, Williams & Hawkes 1995; Thébault *et al.* 2006; Riascos *et al.* 2007). In order to determine the effect of calcein concentration on mean MQI, we therefore modeled mean MQI as a function of calcein concentration while controlling for mean final shell height per sub-site using the main terms and the two-way interaction (this test could not be done with initial shell height as covariate because initial shell height was only obtained for marked individuals). Additionally, growth rate was modeled as a function of calcein concentration and initial shell height using the main terms and the two-way interaction, as shell growth generally decreases with size (Day, Williams & Hawkes 1995; Thébault *et al.* 2006; Riascos *et al.* 2007). Both density (m^{-2}) and final shell height (mm) were modeled as a function of calcein concentration. Body mass (AFDM in mg) was modeled as a function of calcein concentration and final shell height using the main terms and the two-way interaction. AFDM and final shell height were log-transformed because mass is scaled exponentially with height.

The term calcein concentration was always fitted as a categorical variable as we expected a threshold concentration at which detrimental effects would occur. All other variables were fitted as continuous variables. Due to the nested structure of the data, site and, where applicable, sub-site (nested in site) were used as random effects. As such, any difference in environmental conditions and calcein immersion time between sites was included in the analysis as part of the random effect of site. Assumptions of normality and homogeneity of residuals were met for all models. Model selection was based on likelihood ratio tests, following a step-down approach as described in West *et al.* (2006). All analyses were done in R (R Development Core Team 2008; version 2.10.0) using the package 'nlme' (Pinheiro *et al.* 2008).

At the start of this experiment a parallel experiment on *L. lacteus* was carried out in the same study area in order to test whether the administration of 500 ml of a relatively low concentration of calcein (200 mg l^{-1}) for 2 h would affect density and mean shell height. This parallel study was carried out at 11 paired control and calcein-treated sites, which were sampled in April 2008, three months after adding the calcein solution (just as in the main experiment).

Results

In the parallel experiment, a paired *t*-test showed no significant differences in densities between the control and calcein-treated (200 mg l^{-1}) sites ($t = 0.539$, $df = 10$, $P = 0.60$). Additionally, no significant differences in mean shell height were observed between the paired control and calcein-treated sites ($t = 0.516$, $df = 8$, $P = 0.62$). This suggests that application of 500 ml of a concentration of calcein as low as 200 mg l^{-1} does not affect mortality and/or size-selective migration of *L. lacteus*. On the basis of this it seems justified to assume that an even lighter calcein concentration of 100 mg l^{-1}, as used in our main experiment, will have negligible effects on mortality and/or migration of *L. lacteus*.

In the main experiment, calcein produced clearly visible fluorescent marks in shells of *L. lacteus* at all concentrations (Fig. 4.2). The most parsimonious model for estimating mean MQI contained only the main term mean final shell height and no effect of calcein concentration, with mean MQI increasing for decreasing mean final shell height. This model was significantly better than the intercept-model ($L = 7.19$, $df = 1$, $P = 0.01$). Although not significant, there seems to be a trend that MQI increased for sites that were treated with higher calcein concentrations (Fig. 4.2 and Table 4.1 for the estimated parameters of the model with the main term calcein concentration still included). That marking success depends on shell size (i.e., age) is also indicated by Fig. 4.3, which shows that the percentage successfully marked (MQI >1) is especially high for individuals with a final shell height smaller than 7.5 mm (varying from 53% to 74%), but decreases rapidly for individuals larger than 7.5 mm (varying from 0% to 22%).

Growth rate, controlled for initial shell height, did not differ significantly between calcein concentrations (Table 4.2). The best model, with only the main term initial shell height, compared with the intercept-model without this term, indicated that the effect of initial shell height on growth rate was highly significant ($L = 21.41$, $df = 1$, $P < 0.001$), although the percent variability explained was low ($R^2_{LR} = 0.08$). Individual daily growth rate ranged between 4.7 µm day^{-1} and 56.6 µm day^{-1} and decreased with size ($y = -1.586\ x + 30.856$, $N = 261$; Fig. 4.4).

The model with the main term calcein concentration and an intercept-model without this term, indicated a nearly significant effect of calcein concentration on density of *L. lacteus* ($L = 7.24$, $df = 3$, $P = 0.07$). This effect is largely due to the drop in densities at sites treated with the highest calcein concentration (800 mg l^{-1}; Fig. 4.5), which was just significantly lower than at sites treated with the lowest concentration of 100 mg l^{-1} (Table 4.3; $t = -2.105$, df = 18, $P = 0.049$).

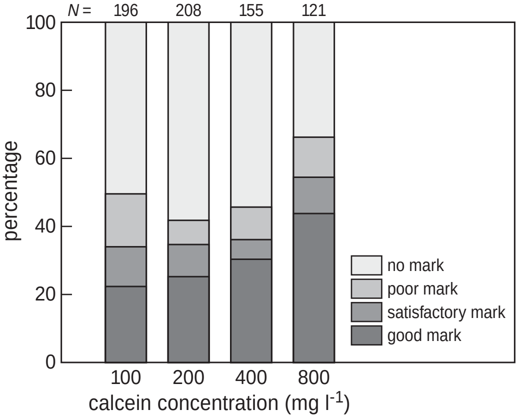

Figure 4.2 Plotted as a function of calcein concentration, the percentage of *Loripes lacteus* individuals with respectively no mark (MQI = 0), a poor quality mark (MQI = 1), a satisfactory quality mark (MQI = 2), and a good quality mark (MQI = 3). Sites are pooled.

Table 4.1 Estimated parameters from the two-way mixed model ANOVA with mean MQI per sub-site as dependent variable, calcein concentration as fixed effect, mean final shell height per sub-site as covariate and site as random effect. We assumed that the random effect of site only acts on the intercept. The random effect representing the between-site variance is 0.132. The estimated value for the residual variance is 0.552.

	Value	SE	*df*	*t*-value	*P*-value
(Intercept)	4.357	1.245	17	3.499	0.003
Mean final shell height (mm)	-0.443	0.167	17	-2.649	0.017
Factor (calcein conc. 200 mg l^{-1})	0.090	0.297	17	0.302	0.766
Factor (calcein conc. 400 mg l^{-1})	0.067	0.296	17	0.227	0.823
Factor (calcein conc. 800 mg l^{-1})	0.583	0.294	17	1.981	0.064

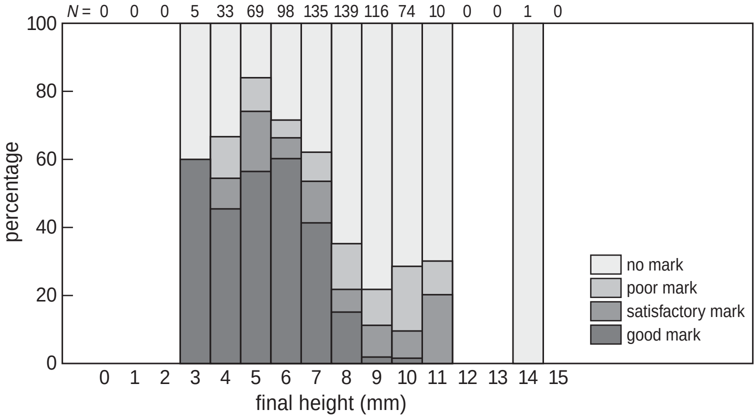

Figure 4.3 Plotted as a function of final shell height (mm), the percentage of *Loripes lacteus* individuals with respectively no mark (MQI = 0), a poor quality mark (MQI = 1), a satisfactory quality mark (MQI = 2), and a good quality mark (MQI = 3).

Table 4.2 Estimated parameters from the two-way mixed model ANOVA with growth rate (µm day^{-1}) of *Loripes lacteus* as dependent variable, calcein concentration as a fixed effect, initial shell height (mm) as a covariate and site and sub-site (nested within site) as random effects. We assumed that the random effect of site only acts on the intercept. The random effect representing the between site variance is 3.91^2 and the random effect representing the between sub-site variance is 1.89^2. The estimated value for the residual variance is 7.68^2.

	Value	SE	*df*	*t*-value	*P*-value
(Intercept)	31.172	2.433	232	12.811	<0.001
Initial shell height (mm)	-1.608	0.345	232	-4.657	<0.001
Factor (calcein conc. 200 mg l^{-1})	-0.029	1.834	18	-0.016	0.988
Factor (calcein conc. 400 mg l^{-1})	0.264	1.834	18	0.144	0.887
Factor (calcein conc. 800 mg l^{-1})	-1.136	1.832	18	-0.620	0.543

Table 4.3 Estimated parameters from the one-way mixed model ANOVA with density (m^{-2}) of *Loripes lacteus* as dependent variable, calcein concentration as fixed effect and site as random effect. We assumed that the random effect of site only acts on the intercept. The random effect representing the between site variance is 701.4^2. The estimated value for the residual variance is 523.6^2.

	Value	SE	*df*	*t*-value	*P*-value
(Intercept)	1547.9	330.8	18	4.679	<0.001
Factor (calcein conc. 200 mg l^{-1})	86.4	279.9	18	0.309	0.761
Factor (calcein conc. 400 mg l^{-1})	-330.0	279.9	18	-1.179	0.254
Factor (calcein conc. 800 mg l^{-1})	-589.3	279.9	18	-2.105	0.049

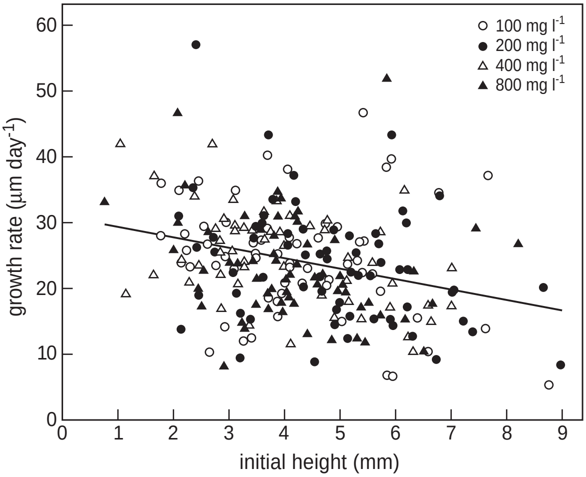

Figure 4.4 Relationship between initial shell height (mm) and daily growth rate (µm day^{-1}) of *Loripes lacteus* with the random intercept effect of site removed. Each symbol represents a different calcein concentration. Line represents the significant mixed model with daily growth rate as the dependent variable and initial shell height as the main term in which the non-significant effect of calcein treatment has been removed ($y = -1.586\,x + 30.856$, $R^2_{LR} = 0.08$, $P < 0.001$, $N = 261$).

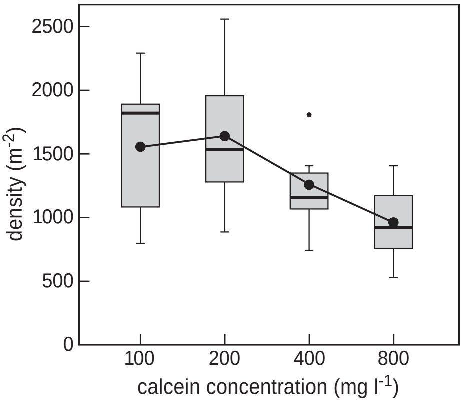

Figure 4.5 Density (m^{-2}) of *Loripes lacteus* at various calcein concentrations with the random intercept effect of site removed. Sample size for each treatment is $N = 7$. Box-and-whisker plots give the mean (large dot), median (horizontal line inside the box), interquartile range (box), range (bars), and outliers (small dot).

There were no differences in size-frequency distribution of *L. lacteus* between calcein treatments, with heights ranging from 3 to 14 mm (Fig. 4.6). The model with the main term calcein concentration and an intercept-model without this term, indeed indicated the absence of an effect of calcein concentration on final shell height ($L = 2.86$, $df = 3$, $P = 0.41$; site and sub-site (nested within site) were included as random effects in each model).

There was no evidence that body condition ([$\log_{10}$ (AFDM)] controlled for the covariate [$\log_{10}$ (final height)]) varied between calcein concentrations (Table 4.4). Comparison of this model with an intercept-model without the main term calcein concentration, indicated the absence of an effect of calcein concentration on body condition ($L = 1.02$, $df = 3$, $P = 0.80$).

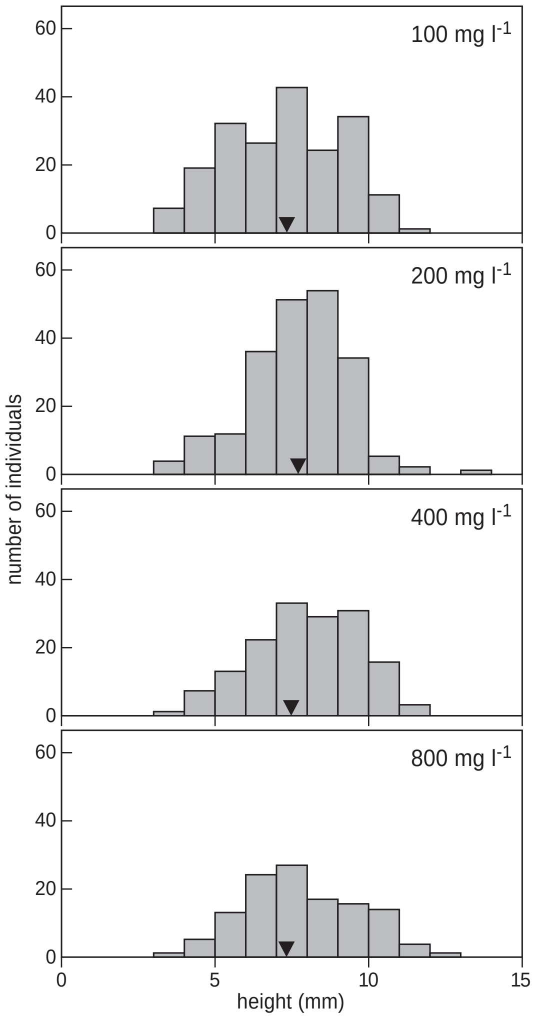

Figure 4.6 The size-frequency distribution of *Loripes lacteus* plotted per calcein treatment (100 mg l^{-1}, 200 mg l^{-1}, 400 mg l^{-1} and 800 mg l^{-1}). Sites are pooled. Per calcein treatment, the mean height is indicated by a black arrow.

Table 4.4 Estimated parameters from the two-way mixed model ANOVA with log-transformed AFDM in mg [$\log_{10}(x)$] as dependent variable, calcein concentration as fixed effect, log-transformed height [$\log_{10}(x)$] as covariate and site and sub-site (nested within site) as random effects. We assumed that the random effects of site and sub-site only act on the intercept. The random effect representing the between-site variance is 0.000015^2 and the random effect representing the between sub-site variance is 0.12^2. The estimated value for the residual variance is 0.15^2.

	Value	SE	*df*	*t*-value	*P*-value
(Intercept)	-4.315	0.072	646	-60.823	<0.001
Log-transformed height [$\log_{10}(x)$]	2.456	0.059	646	41.353	<0.001
Factor (calcein conc. 200 mg l^{-1})	0.003	0.067	18	0.046	0.964
Factor (calcein conc. 400 mg l^{-1})	0.033	0.067	18	0.492	0.629
Factor (calcein conc. 800 mg l^{-1})	-0.069	0.067	18	-1.023	0.320

Discussion

Immersion of a seagrass-covered patch with a calcein solution ranging from 100 to 800 mg l^{-1} for approximately 2 h was sufficient to produce a bright fluorescent mark on both the in- and outside of shells of *L. lacteus* that live burrowed in muddy seagrass beds. These results corroborate earlier fluorochrome studies demonstrating that immersion in calcein solutions of 100–150 mg l^{-1} were sufficient to mark the shells of gastropods (Moran 2000; Riascos *et al.* 2007) and bivalves (Thébault *et al.* 2006; Riascos *et al.* 2007; Herrmann *et al.* 2009). There was a tendency for marking success to increase with higher calcein concentrations (ranging from 34% to 55%; Fig. 4.2).

Our results suggest an age-dependent effect on the success of calcein marking because small (i.e., young) lucinids were significantly better marked than large ones (Fig. 4.3 and Table 4.1). Age-dependent shell marking success has also been observed in abalones (Haliotidae; Day, Williams & Hawkes 1995; Riascos *et al.* 2007), scallops (Pectinidae; Thébault *et al.* 2006), and surf clams (Mactridae; Riascos *et al.* 2007). Moreover, growth after marking decreased significantly with shell size (Fig. 4.4). This inverse relationship reflects a decrease in the rate of shell accretion throughout the life span, an ontogenetic change widely recognized in the life history of many organisms that grow by accretion (e.g. Harrington 1989). Low growth rates could reduce the transfer of calcein through the mantle epithelium (Day, Williams & Hawkes 1995): a 2-h exposure to calcein was probably insufficient to produce an internal fluorescent mark in the larger lucinids.

Nevertheless, there may be other reasons why smaller individuals are more successfully stained. Burrowing depth of *L. lacteus* tends to be a function of size, with larger individuals living more deeply burrowed compared to smaller individuals (pers. obs.). Therefore, one could suggest that being more deeply burrowed, larger lucinids might have less access to the calcein solution allocated, which could result in a decrease in marking success. However, we consider this option as rather unlikely, as even deeply burrowed lucinids have access to the overlying water by means of an anterior inhalant tube, which is used to pump oxygen-rich water to their gills (Allen 1958; Taylor & Glover 2000).

In this study, calcein immersion of a 0.071 m^2 surface area was sufficient to collect marked specimen after an intervening period of three months. The fact that we also collected individuals that were not marked, could partly be explained by local dispersal of unmarked individuals from the surrounding area that moved into the immersed study site after allocation of the calcein solution. To circumvent this problem we suggest the immersion of larger surfaces. Given the relatively low percentage of marked individuals, the method described in this study seems particularly suitable for burrowing bivalves of which natural densities are relatively high, as is the case for *L. lacteus*.

Moran (2000) argues that if inconsistent mark incorporation is observed, then researchers should consider whether excluding unmarked individuals might bias experimental results. In this study, we observed a size-dependent effect on marking success, with less large individuals being marked compared to small ones. If growth rate does not differ within size classes, a size-dependent effect on marking success will not be a constraint to accurately estimate growth for *L. lacteus*, given that growth data are obtained for the full-size range. However, if we assume variation in growth rate between individuals of the same size-class and that slow growing individuals of a certain size-class are less likely to be marked, then this could result in an overestimation of growth for the lucinid population. Indeed, it seems that there is much variation in growth rate within a size-class and the minimum growth rate necessary to be successfully marked is in the order of 8 µm day^{-1} (Fig. 4.4). As the minimum growth of especially large (i.e., old) individuals could be lower than 8 µm day^{-1}, overestimation of growth seems more of a problem for larger individuals. However, even if the observed growth rates for large individuals is overestimated, the fact that production by large individuals is relatively low compared to smaller ones suggests that our marking method seems appropriate for estimating secondary production of the full population of *L. lacteus*.

In our study, the immersion time was restricted due to the tidal cycle, as immersion took place *in situ* during low tide and lasted until the moment that the study site was flushed by the incoming tide. To avoid major differences in immersion time between sites, immersion time for all sites was set by the site that was emerged for the shortest period. As a consequence, we used a relatively short immersion time 2.1 ± 0.4 h (mean ± SD) compared to other fluorochrome studies where minimum immersion times varied from 3 h (Thébault *et al.* 2006; Riascos *et al.* 2007; Herrmann *et al.* 2009) to 12 h (Day, Williams & Hawkes 1995). The advantage of a relatively short immersion time is that the period of potential stress to the lucinids is limited. On the contrary, lucinids that are immersed for a relatively short period, have less time to incorporate the stain into their shell, which could result in a decrease in marking success.

Kaehler and McQuaid (1999) encountered problems when *in situ* immersing mussels in calcein solution as, apart from the growing edge, other parts of the outer calcareous layer were also fluorescently marked probably due to deposition of $CaCO_3$ by microbial endoliths within the shell, making the identification of a distinct growth mark difficult. In this study, *in situ* immersion of burrowed lucinids in calcein solution resulted in clear well-defined fluorescent growth marks on the exterior of the shell, which might imply that shells of *L. lacteus* are less infested by cyanobacterial shell-borers. This presumed low endolith infestation could be a result of the burrowing life-style of *L. lacteus*, suggesting that this immersion method is especially suitable for marking endobenthic marine organisms.

During immersion, we encountered no problems with calcein solution leaking from the enclosed area, which is likely to be a result of the fine-grained sediment which is typical for the seagrass habitat in our study area. The small grain sizes also prevented the solution from rapid drainage into the sediment. However, when applying this immersion technique in more granulose sediments, leaking and rapid drainage of the calcein solution could become more of a problem. In this case, it is probably better to use a benthic chamber with a closed-circuit flow of the calcein solution as designed by Thébault *et al.* (2006).

Identification of possible lethal and sub-lethal effects of *in situ* calcein marking is an obvious requirement to assess the suitability of this staining method in bivalve growth studies, especially if growth rate would be slowed down by applying this method. Although not significant, we observed a tendency for *L. lacteus* densities to decrease when applying calcein concentrations ≥400 mg l^{-1} (Fig. 4.5). As we cannot exclude the possibility that detrimental effects of calcein resulted in increased mortality, care should be taken when applying such high calcein concentrations to mark lucinids. That we observed no detrimental effects of calcein concentration on growth rates agrees with the studies of Rowley and MacKinnon (1995), Moran and Marko (2005) and Riascos *et al.* (2007). Just as concluded by Riascos *et al.* (2007) for a gastropod species (*Concholepas concholepas*) and a bivalve species (*Mesodesma donacium*), calcein concentration had no detectable negative effects on body condition in *L. lacteus*. Size-frequency distribution did not significantly differ between calcein treatments (Fig. 4.6), which suggests that the effect of calcein concentration on local dispersal is limited.

Traditionally, mark-and-recapture techniques used for studying growth were deemed unsuitable for fragile burrowing bivalves as the physical handling and transportation involved would affect growth and survival. That we detected no detrimental effects of our marking method when using calcein concentrations ≤200 mg l^{-1} indicates that *in situ* marking by the fluorochrome calcein provides a suitable growth marker for *L. lacteus* (and potentially other fragile burrowing bivalves). However, as marking success decreased with age, our marking method seems less suitable for bivalve populations that mainly exist of older individuals.

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Chapter 5

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

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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.

Introduction

In the current biodiversity crisis, predators have often been the ones disappearing first (Byrnes, Reynolds & Stachowicz 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 & Worm 2003) and overhunted (Johnson, Isaac & Fisher 2007). As predators often play key roles in the structuring and organisation 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 & 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 & Pascual 2006). This matches earlier conclusions that predators switching diet promote prey coexistence (Murdoch 1969). More generally, it can be stated that *adaptive* behavior by predators enhances the stability in systems where otherwise one prey species outcompetes another (Fryxell & 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 & 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 & 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, Morrison & Hedges 2000). There are some well-known examples of how predators affect the abundances of their prey *and* the prey's resources (Estes & Palmisano 1974; Ripple & 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, Diouris & Le Pennec 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 molluscs, 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 mollusc-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 on these prey.

Are red knots specialist or generalist predators?

In general, plotting so-called 'depletion trajectories' enables exploring the diet strategy applied by the predator (Brown & Mitchell 1989) (Fig. 5.1). 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. 5.1A) (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. 5.1B; Holt & 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.

In this framework, regressions of GUD against initial prey density (IPD) should be diagnostic for the diet strategy applied by the predator (Fig. 5.2). Considering the specialist predator and the prey that it specializes on, GUD will be constant and independent of IPD above a certain IPD (Fig. 5.2A), whereas GUD and IPD will be similar in the prey type that it ignores (Fig. 5.2B). Hence, there will be no relation between the GUD on prey type 1 and

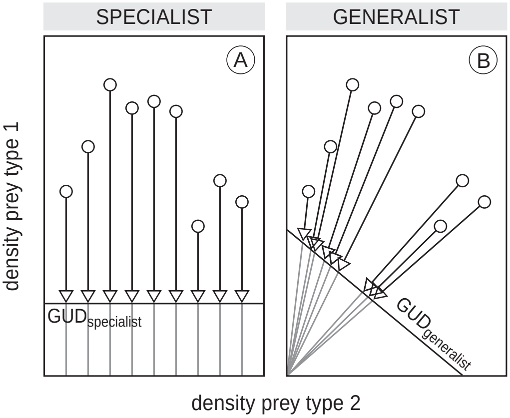

Figure 5.1 (A) Specialist predators only feed on a single prey type and will deplete it down (arrows) to a fixed giving-up density GUD (horizontal line). (B) By contrast, generalist predators feed on multiple prey types, in the present case two. Both prey types will be depleted down to a combination 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 & Kotler 1987).

the GUD on prey type 2 (Fig. 5.2C; comparable to Fig. 5.1A). By contrast, in the generalist predator there will be much variation in the GUD on prey type 1 (Fig. 5.2D) as well as on type 2 (Fig. 5.2E), 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. 5.2F; comparable to Fig. 5.1B).

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./m^2) on the prey that the specialist knot feeds on:

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

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 & Mitchell 1989), we see that in the generalist knot, the GUD on *Dosinia* ($\mathrm{GUD_{Dos}}$) depends on the GUD on *Loripes* ($\mathrm{GUD_{Lor}}$) (and *vice versa*):

$$\mathrm{GUD_{Dos}} = \frac{\mathrm{QHR}}{e_{\mathrm{Dos}}a - \mathrm{QHR}ah} - \frac{\mathrm{GUD_{Lor}}(e_{\mathrm{Lor}}a - \mathrm{QHR}ah)}{e_{\mathrm{Dos}}a - \mathrm{QHR}ah} \qquad (2)$$

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

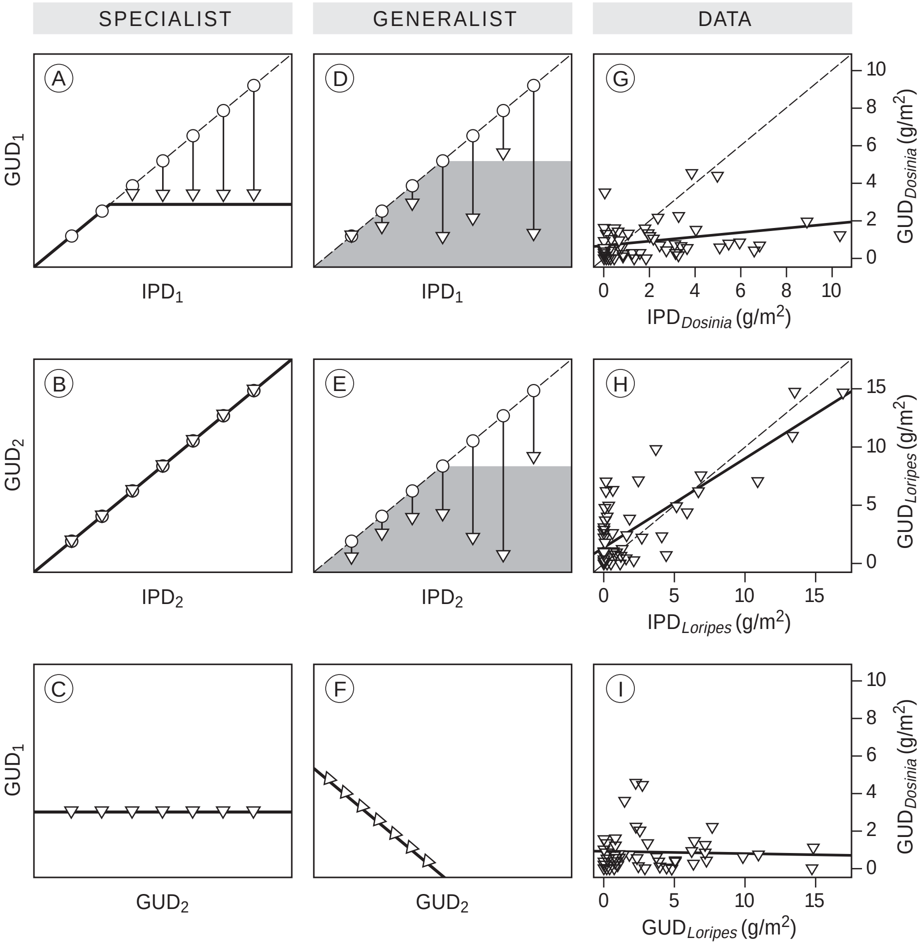

Figure 5.2 Illustrations of different predator dietary strategies. (A) A specialist predator will deplete prey type 1 down to a fixed giving-up density, GUD_1, leaving patches unexploited when the initial prey density $IPD_1 < GUD_1$. (B) It will not feed upon prey type 2; hence $GUD_2 = IPD_2$. (C) 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.

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 of 0.2 mg ash-free dry mass ($AFDM_{flesh}$) 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 & 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 (respectively 2.57 and 7.28 mg $AFDM_{flesh}$). 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 account the average $AFDM_{flesh}$ 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 (19º 53.0' N; 16º 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 provided 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. 5.2), 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 $\text{AFDM}_{\text{flesh}}$ of a subset of individuals. The latter was done by separating flesh from shell and drying it for three days at 60ºC. Next, that dried flesh was weighed (to the nearest 0.1 mg) and incinerated for 5 h at 550ºC, subsequently ash mass was determined (to the nearest 0.1 mg). The resulting $\text{AFDM}_{\text{flesh}}$ (in grams)-to-length (L, in millimetres) relationships (for *Dosinia*, $\text{AFDM}_{\text{flesh}} = 10^{-5.05}L^{3.07}$, $N = 166$ specimens, $R^2 = 0.96$, $P < 0.001$; for *Loripes*, $\text{AFDM}_{\text{flesh}} = 10^{-4.73}L^{2.96}$, $N = 191$ specimens, $R^2 = 0.95$, $P < 0.001$) were used to predict $\text{AFDM}_{\text{flesh}}$ 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 recollected 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 $\frac{dH_t}{dt}$ declines with an increase in size H_t (the shell height at the onset of the experiment in 2009) in the following way:

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

where H_∞ is the mean maximum size and k is the growth constant. For each individual *Loripes* we estimated k by defining $\frac{dH_t}{dt}$ 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 & 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 a 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 of 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, Tomassen & Roelofs 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 onset of the exclosure experiment, i.e., October 2009. In total, we collected 17 dropping samples (more or less equally divided over the seven tidal flats), each consisting of 40 (± 3) droppings on average, which were stored in the freezer. Back in The Netherlands, samples were dried for 3 days at 60ºC; subsequently we determined dry mass (DM_{drop}) of those fragments that retained on a 300-µm sieve, separately for both species (Dekinga & 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 $\overline{DM_{shell}}$ of a consumed prey (Dekinga & Piersma 1993). For this purpose we used regression coefficients of DM_{shell} (in grams)–length (in millimeters) relationships that were determined on specimens collected in Banc d'Arguin in January 2011 (for *Dosinia*, $DM_{shell} = 10^{-3.43}L^{2.63}$, $N = 124$ specimens, $R^2 = 0.96$, $P < 0.001$; for *Loripes*, $DM_{shell} = 10^{-4.19}L^{3.25}$, $N = 119$ specimens, $R^2 = 0.96$, $P < 0.001$; Onrust *et al.* 2013). In a recent calibration study on red knots it was shown that about 65% of the ingested $\mathrm{DM}_{\mathrm{shell}}$ is found back as $\mathrm{DM}_{\mathrm{drop}}$, both for *Dosinia* as well as for *Loripes* (Onrust *et al.* 2013). Therefore, N_{diet}, the number of prey items per species per sample is given by $\mathrm{DM}_{\mathrm{drop}}/(0.65\cdot\overline{\mathrm{DM}_{\mathrm{shell}}})$.

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 was taken at a distance of 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 & Blomert 1992).

We applied Ivlev's 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: $I = (F_{\mathrm{diet}}-F_{\mathrm{avbl}})/(F_{\mathrm{diet}}+F_{\mathrm{avbl}})$. 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 $F_{\mathrm{diet,D}}$ relative to *Loripes* (L) is given by: $N_{\mathrm{diet,D}}/(N_{\mathrm{diet,D}}+N_{\mathrm{diet,L}})$. Similarly, $F_{\mathrm{avbl,D}}$, the relative contribution of *Dosinia* to the available food supply equals $N_{\mathrm{avbl,D}}/(N_{\mathrm{avbl,D}}+N_{\mathrm{avbl,L}})$. 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. 5.2G; $y = 0.66 + 0.12x$, $F_{1,53} = 4.71$, $P < 0.05$). 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 non-significant 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. 5.2A). 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. 5.2H; $y = 1.42 + 0.76x$, $F_{1,53} = 89.59$, $P < 0.001$). 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

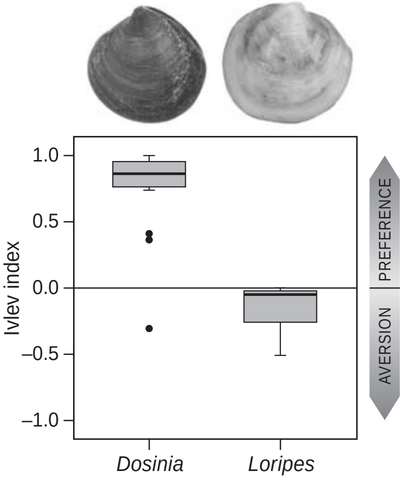

Figure 5.3 Diet selectivity at the onset of the exclosure experiment, October 2009, by means of analyses of red knots feces. red knots (*Calidris canutus*) had a clear preference for *Dosinia* (Ivlev electivity index >0) and a clear aversion of *Loripes* (Ivlev index <0). Box and whisker plots give the median (horizontal line inside the box), interquartile range (box), and outliers (small black dots).

suggests that predation on *Loripes* was marginal (cf. Fig. 5.2B).

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. 5.2I; $y = 0.93 - 0.01x$, $F_{1,53} = 0.07$, $P = 0.79$), which corroborates the prediction for a specialist predator on *Dosinia* (Fig. 5.2C) and refutes the prediction for a generalist predator (Fig. 5.2F).

Fecal analysis

Comparing the relative proportions of *Dosinia* and *Loripes* in the diet with those available in the field yielded a clear result (Fig. 5.3). *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. 5.4; $k_{\text{control}} = 0.66$, $k_{\text{exclosure}} = 0.58$, $t = 2.89$, $P < 0.005$, $N = 10$ pairs, $N = 105$ *Loripes*). The significance of this outcome was insensitive to the assumed value of 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 & J. A. van Gils, *unpublished data*).

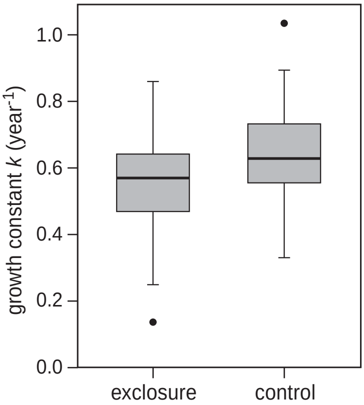

Figure 5.4 *Loripes* grew faster in the controls than in the exclosures, as shown here by boxplots of individual Von Bertalanffy's growth constants *k*. Box and whisker plots give the median (horizontal line inside the box), interquartile range (box), and outliers (small black dots).

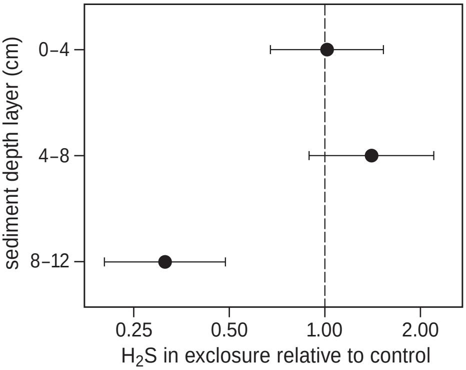

Figure 5.5 Sulfide concentrations were lower in the exclosures than in the controls, but only so in the sulfide-richest deepest layer (8–12 cm). Plotted are means ± SE of the ratio between sulfide in the exclosure and in the paired control, with the dashed vertical line representing the prediction of no difference between exclosure and control.

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. 5.5; $N = 21$ pairs). A linear mixed-effect model with random intercepts showed the following depth-dependent estimates for $\log_{10}$-transformed $H_2S_{excl}/H_2S_{control}$ 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. 5.2 and 5.3). 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 $AFDM_{flesh}/m^2$), 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 dropping analyses, in which the Ivlev index on *Loripes* is significantly above -1 (Fig. 5.3). 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 $AFDM_{flesh}/m^2$, N = 112 benthic samples, which represents only those sites that where resampled in 2010), than one year later, in October 2010 (1.2 ± 0.1 g $AFDM_{flesh}/m^2$, 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* (van Gils *et al.* 2013).

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 AFDMflesh and DMshell), 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 (Oudman *et al.* in revision). 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, Oerkert & Knape 1964). Furthermore, shallow-water fishes and crabs deterred feeding when offered prey which were collected in sulfide-rich deep-sea hydrothermal vents, presumably due to the high sulfide concentrations inside these prey (Kicklighter, Fisher & Hay 2004).

In spite of a 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. 5.4). 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, Diouris & Le Pennec 1994) and in other members of the Lucinidae family (Duplessis *et al.* 2004; Dufour & 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

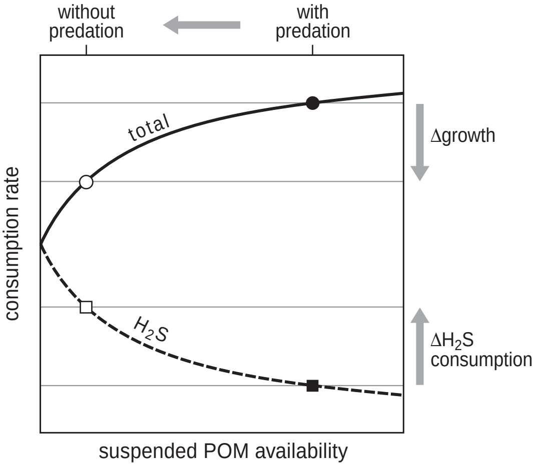

Figure 5.6 Schematic depiction of a possible mechanism showing how *Loripes* may experience hampered growth in the absence of predation on *Dosinia*. Without predation, *Dosinia* densities will be higher, and hence suspended POM (particulate organic matter) availability will be lower. Assuming that in *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 total consumption rate (suspended POM and sulfide), and hence growth rate, will decline.

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 pore-water 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. 5.5). This proposed mechanism can be captured in a simple Holling type II functional response model on two resources, as exemplified in Fig. 5.6. There is ample evidence for interspecific competition among suspension-feeding bivalves (e.g., Peterson & 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, Orth & Duarte 2006), which makes the competition for suspended POM at the seagrass-covered tidal flats of Banc d'Arguin 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 $r = -0.79$, $P < 0.05$, $N = 8$; van Gils *et al.* 2013).

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. 5.5), 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 & 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 shell growth rates, it may be beneficial to other organisms as 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 to 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 (van der Heide *et al.* 2012). 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.

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Chapter 6

Seasonal changes in mollusc abundance in a tropical intertidal ecosystem, Banc d'Arguin (Mauritania): testing the 'depletion by shorebirds' hypothesis

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Abstract At temperate latitudes densities and biomass of intertidal molluscs tend to be strongly seasonal. Here we provide a comparative study on seasonality of bivalves and gastropods in the tropical intertidal seagrass-covered soft sediment environment of Banc d'Arguin, Mauritania (20°N, 16°W). In this system, benthivorous shorebirds exert considerable predation pressure with strong seasonal variations. It has been proposed that during the period when (adult) shorebirds are absent (May–August) benthic biomass would be able to recover, but a first test was inconclusive. Over a full year (March 2011–February 2012), each month we sampled benthic invertebrates at sixteen permanent sites. The total of 3,763 specimens comprised 20 species, representing eight orders and 19 families. Bivalves were much more common than gastropods. The bivalve *Loripes lucinalis* dominated the assemblage throughout the year (58% of total number), followed by *Dosinia isocardia* (10%), *Senilia senilis* (8%) and the gastropod *Gibbula umbilicalis* (6%). Average biomass amounted to 32 g AFDM m^{-2}, of which the large West-African bloody cockle *Senilia* made up three-quarter, *Loripes* 16%, *Gibbula* 2% and *Dosinia* 1%. Across the 20 species, lowest densities were reached in late spring (May) and summer (Aug.), whereas highest densities occurred in autumn (Oct.). The lowest overall density of 676 specimens m^{-2} in August more than doubled to a peak density of 1,538 specimens m^{-2} in October, most of the increase being due to strong recruitment in both *Loripes* (densities increasing from 322 specimens m^{-2} in Sept. to 785 specimens m^{-2} in Oct.) and *Dosinia* (densities increasing from 18 specimens m^{-2} in Aug. to 265 specimens m^{-2} in Sept.). Our results suggest that by the time the feathered molluscivore predators returned in high numbers to Banc d'Arguin (after their summer breeding season in the Arctic), benthic animals were at a peak. In order to quantitatively understand the seasonal changes in mollusc abundance, we build upon a recently published optimal diet model in which the most abundant molluscivore shorebird, the red knot (*Calidris canutus*), could choose between *Loripes* and *Dosinia*. Observed changes in densities of these two bivalves closely match depletion trajectories predicted by the model. We conclude that molluscivore shorebirds are able to deplete their food stocks in the course of their 'winter' in a tropical intertidal area.

Introduction

It is well established that benthic invertebrates living in temperate intertidal soft-sediment systems show pronounced seasonality in density and biomass (Beukema 1974; Zwarts, Blomert & Wanink 1992; Zwarts & Wanink 1993; Urra *et al.* 2013). In the tropics, temperature fluctuations are less pronounced, and this may also be true for the seasonal changes in food availability for benthic invertebrates (Sastry 1979; Alongi 1990; Lubet 1994; Mandal & Harkantra 2013). This could lead to tropical intertidal benthic communities remaining rather stable throughout the year (Piersma 1982; Wolff 1991; Dittmann 2000), even in regions with distinct rainy and dry seasons (Silva *et al.* 2011). However, benthic invertebrates are the food of consumers such as crabs, shrimp, fish and shorebirds (van de Kam *et al.* 2004). The shorebird component of this predator community is highly seasonal by virtue of the fact that shorebirds breed in northern areas from which they migrate south to winter in coastal wetlands, e.g., along the coasts of West-Africa (van de Kam *et al.* 2004; Piersma 2007). This would result in strongly seasonal predation pressure (Wolff & Michaelis 2008).

Such seasonality in predation pressure could be strong in Banc d'Arguin (Mauritania, West-Africa), which is an area of over 10,000 km^2 of shallow inshore waters bordering the Sahara (Wolff *et al.* 1993a). This area has received protection from human overexploitation by the establishment in 1976 of the Parc National du Banc d'Arguin (PNBA). PNBA is one of the most important waterbird areas on the West-African coastline, hosting over two million wintering shorebirds (Engelmoer *et al.* 1984; Smit & Piersma 1989; Ens *et al.* 1990; Zwarts *et al.* 1990; Zwarts *et al.* 1998), of which the red knot *Calidris canutus* is by far the most numerous molluscivore (230,000–300,000 individuals), followed by the much less abundant oystercatcher *Haematopus ostralegus* (5,000–8,000 individuals; Zwarts *et al.* 1998; Hagemeijer *et al.* 2004; Diawara & Overdijk 2007). More than 3/4 of the intertidal flats at Banc d'Arguin are covered by seagrass beds (Wolff & Smit 1990), which, compared to bare flats, support the highest densities of shorebirds (Altenburg *et al.* 1982). This is for good reasons, since seagrass habitat is among the most productive and richest of shallow marine habitats, both locally (Honkoop *et al.* 2008) as well as globally (Duarte & Chiscano 1999).

During the first studies of shorebirds and benthos of the Banc d'Arguin in 1980, the high ratio of birds to standing benthic biomass, in comparison with ratios found elsewhere in intertidal soft-sediment areas, were unexpected (Altenburg *et al.* 1982; Engelmoer *et al.* 1984). The high ratio implied high secondary production, but also suggested that shorebirds would be able to substantially reduce the standing stocks of benthos in the course of the main season of predation, i.e., from September through April. Wolff and Michaelis (2008) put this to a test by comparing the benthic biomass sampled across an extensive area in September 1988 (i.e., at the start of a shorebird migration season) with the standing stocks in February–March 1986 (i.e., at the end). The lack of difference between the two values may be explained either by an absence of depletion of benthos during the season of shorebird predation, or because of differences between years obliterated the measurement of the proposed effect. Here we provide a stronger test of the seasonal depletion hypothesis by examining monthly changes over a full year in a seagrass bed, intensively used by shorebirds, in a nearshore site of the Banc d'Arguin. As molluscs are by far the largest component of the intertidal benthic community of Banc d'Arguin (e.g., Piersma 1982; Wolff *et al.* 1993a; Michaelis & Wolff 2001), and since molluscivorous shorebirds, especially red knots, are very numerous, we limited our study to bivalves and gastropods.

Materials and methods

Study area

This study was carried out from the PBNA Scientific Station at Iwik Peninsula on a nearshore area of dense seagrass beds (mainly *Zostera noltii* Hornem.), locally known as Abelgh Eiznaya (Fig. 6.1; see also Leyrer *et al.* 2006). 16 stations were chosen randomly within a 200-m radius from a central point (19°53.40'N, 16°18.50'W), which were sampled at monthly intervals from March 2011 to February 2012. About 1/3 of the stations was located in dense seagrass habitat, 1/3 in intermediate seagrass and 1/3 in bare habitat, with fine (median grain size ~50 µm), intermediate (~100 µm) and coarse sediment texture (~150 µm), respectively (Folmer *et al.* 2012). Tidal regime is the usual M2 type, also known as the 'principal lunar semi-diurnal' type, with an average amplitude of 1.61 m (Wolff & Smit 1990). Meteorological measurements at a site 65 km south of the study area (at Nouamghar; http://www.accuweather.com/fr/mr/nouamghar/1112585/month/1112585?) showed monthly variations in minimal (15.0–25.7ºC) and maximal (29.0–37.8ºC) daily air temperatures during our study period, with lowest minimum in February and highest maximum in October. Most rain fell in August (27 mm vs. 3 mm/month on average).

Field sampling and laboratory analyses

Each month at each station we took a single sample down to a depth of 20 cm using a core with a 15 cm diameter. In order to determine whether prey were within reach of the 3.5 cm long bill of red knots (Zwarts, Blomert & Wanink 1992), we separately sieved the top layer (0–4 cm) from the bottom layer (4–20 cm), using a 1-mm meshed sieve. The material remaining on the sieve was put in separate labelled plastic bags and frozen within a few hours after sampling. Over subsequent days all fleshed bivalves and gastropods were sorted from the debris of dead shells and seagrass material. Specimens were identified to species level, their lengths were measured to the nearest 0.1 mm, and they were then fixed in buffered formaldehyde (4%). Subsequently (May 2012), biomass of the most abundant species (*Diplodonta circularis*, *Dosinia isocardia*, *Gibbula umbilicalis*, *Loripes lucinalis*, *Mesalia mesal*, *Modiolus* sp., *Petricola pholadiformis*, *Senilia senilis* and *Tellina distorta*) was determined in the laboratory at NIOZ as ash-free dry mass (AFDM). In order to do so, specimens were dried at 60°C for at least 48 hours and were afterwards incinerated at 560°C for 2 hours (bivalves without shells, gastropods with shells).

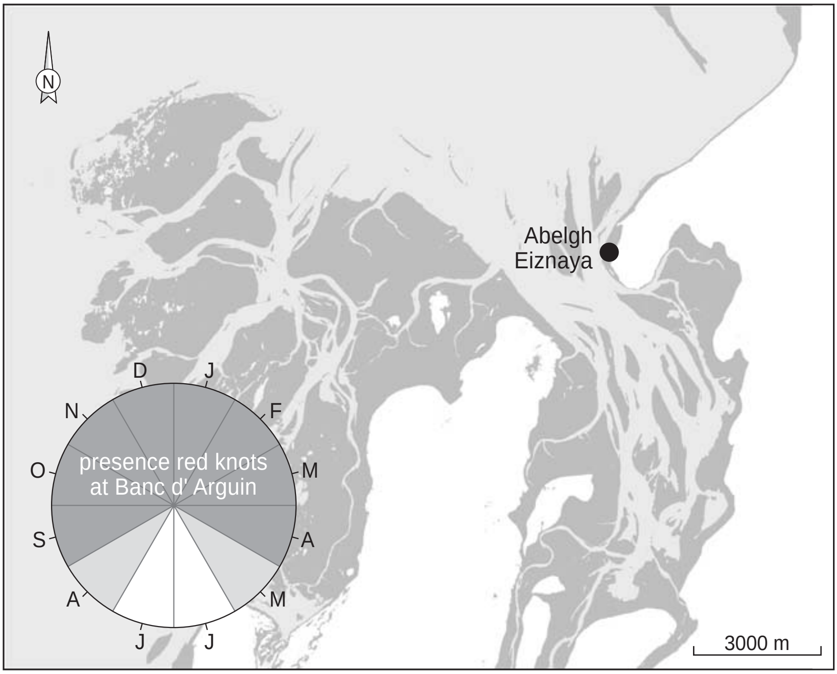

Figure 6.1 Map of the Banc d'Arguin, with tidal flats given in dark grey, ocean in light grey and land in white. Black dot indicates the centre of our study area, Abelgh Eiznaya. Scale bar is expressed in meters. The pie indicates the presence of red knots in Banc d'Arguin (both adults and young birds present during September–April; adults are leaving and arriving in May and August, respectively; only young birds present during June–July). Note that the sampling area of Wolff and Michaelis (2008) was much larger than ours and covered roughly all tidal flats plotted in this map.

Depletion model

We used an existing optimal diet model that has been developed for the most abundant molluscivore shorebird present in our study area, the red knot (van Gils *et al.* 2013). Red knots ingest their prey whole, and hence prey that have a circumference larger than their gape cannot be ingested (Zwarts & Blomert 1992). Most mollusc species found at Banc d'Arguin do not grow very large and therefore large fractions are ingestible for red knots (Piersma, de Goeij & Tulp 1993).

We only modelled the depletion of *Loripes lucinalis* and D*osinia isocardia* and left out all the other mollusc species. This is because *Loripes* and *Dosinia* are the two most abundant molluscs in the system. The third most abundant mollusc, *Senilia senilis*, is a thick-shelled bivalve which grows up to heights of 80 mm and is therefore almost entirely unavailable to red knots (Wolff *et al.* 1987). Our focus on *Loripes* and *Dosinia* only is also justified because these two species together represent 80% of the molluscs consumed by red knots at Banc d'Arguin (van Gils *et al.* 2012; Onrust *et al.* 2013; van Gils *et al.* 2013).

Even though *Loripes* is by far the most abundant bivalve and has a very thin shell, we know that it is not the most preferred prey of the two (van Gils *et al.* 2012; van Gils *et al.* 2013; Oudman *et al.* in revision). Recent evidence suggests that this is due to the specialized metabolism of *Loripes* that lives in symbiosis with chemoautotrophic bacteria inside its gill structures (Herry, Diouris & Lepennec 1989). Feeding experiments with captive red knots showed that they develop diarrhoea, dehydrate, and lose appetite when on a monospecific diet of *Loripes* (Oudman *et al.* in revision). The depletion model used here takes account of the long-term maximum intake rate that red knots can sustain when foraging on *Loripes*. This model, called the Toxin Digestive Rate Model (TDRM), not only explained diet choice differences between years, it also explained most of the variation in annual survival rates in an 8-year study of red knot demographics (van Gils *et al.* 2013). The model is an extension of the so-called Digestive Rate Model (DRM) that explained diet choice in red knots feeding on non-toxic prey (van Gils *et al.* 2005a) and we refer to van Gils *et al.* (2013) for its full mathematical details; here we will only introduce the basics.

According to Holling's functional response model (Holling 1959), energy intake rate Y on two prey types (labelled 1 and 2) is given by:

$$Y = \frac{p_1 a_1 N_1 e_1 + p_2 a_2 N_2 e_2}{1 + p_1 a_1 N_1 h_1 + p_2 a_2 N_2 h_2}, \tag{1}$$

where p is the acceptance probability upon encounter, a is searching efficiency, N is available numerical prey density, e is energy value and h is handling time for respectively prey type 1 and 2. Under some circumstances Y can be maximized by not accepting every prey that is encountered. Diet selection models make predictions about the values of p_1 and p_2 in order to maximize Y.

The classical diet model (Stephens & Krebs 1986), sometimes called the contingency model (CM), ranks prey types on the basis of profitability, i.e., $\frac{e_1}{h_1} > \frac{e_2}{h_2}$. A forager should only accept prey type 1 ($p_1 = 1$) and neglect prey type 2 ($p_2 = 0$) when the energy intake rate on prey type 1 alone exceeds the profitability of type 2, i.e., when

$$\frac{a_1 N_1 e_1}{1 + a_1 N_1 h_1} > \frac{e_2}{h_2}. \tag{2}$$

If not, it should accept both prey types in its diet ($p_1 = 1; p_2 = 1$).

The CM ignores digestive processing constraints, which real-world foragers often face, especially the ones such as molluscivores feeding on bulky prey. The digestive rate model (DRM), developed by Hirakawa (1995) and successfully applied to red knots (van Gils *et al.* 2005a; van Gils *et al.* 2005b; Kraan *et al.* 2009; Quaintenne *et al.* 2010), does take account of such a constraint. Defining k as a prey type's indigestible bulk mass, ballast intake rate X is defined as

$$X = \frac{p_1 a_1 N_1 k_1 + p_2 a_2 N_2 k_2}{1 + p_1 a_1 N_1 h_1 + p_2 a_2 N_2 h_2}. \tag{3}$$

Defining c as the maximum rate at which bulky material can be processed by a forager's digestive system, the following condition should always be met,

$$X \leq c\ . \tag{4}$$

The optimal solution for p_1 and p_2 can either be found graphically or analytically and we refer to the original paper by Hirakawa (1995) or to the first application in red knots by van Gils *et al.* (2005a).

Finally, the TDRM not only takes account of a constraint on processing bulky food, it also includes a maximally tolerable toxin intake rate. Defining s as a prey type's toxin content, toxin intake rate Z is given by:

$$Z = \frac{p_1 a_1 N_1 s_1 + p_2 a_2 N_2 s_2}{1 + p_1 a_1 N_1 h_1 + p_2 a_2 N_2 h_2}\ . \tag{5}$$

Defining q as the maximally tolerable toxin intake rate, the following condition should always be met:

$$Z \leq q\ . \tag{6}$$

Parameters used (Table 6.1) are as in van Gils *et al.* (2013), except for energy values e and ballast masses k, which are estimated in the current study. These parameters, in combination with the relatively low densities of *Dosinia* found in this study (see section 'Results' below), simplify the mathematics of the TRDM. Namely, on all monthly average densities of *Loripes* and *Dosinia* found in this study, red knots would never face a digestive constraint c but always a toxin constraint q van Gils *et al.* (2013) reveal that for the current parameter settings digestive constraint c occurs above 365 available *Dosinia* m^{-2}, while toxin constraint q is reached above 49 available *Loripes* m^{-2}). This implies that all available *Dosinia* can be accepted ($p_D = 1$), while only a proportion of the available *Loripes* can be accepted (i.e., $p_L < 1$ as long as densities are above 49 specimens m^{-2}). This latter proportion p_L can be calculated by assuming a toxin intake rate that is maximally tolerable, i.e.,

$$Z = q\ . \tag{7}$$

Because only *Loripes* is toxic ($s_L = e_L$) and *Dosinia* is not ($s_D = 0$), and because $p_D = 1$, this can be written as

$$\frac{p_L a_L N_L s_L}{1 + p_L a_L N_L h_L + a_D N_D h_D} = q. \tag{8}$$

Rewriting this, p_L equals:

$$p_L = \frac{q(1 + a_D N_D h_D)}{a_L N_L (s_L - q h_L)}\ . \tag{9}$$

Because depletion is expressed as changes in prey *numbers*, we need to calculate the *numerical* intake rate R given by Holling's functional response, which on *Loripes* equals (with p_D falling out since it equals 1):

$$R_L = \frac{p_L a_L N_L}{1 + p_L a_L N_L h_L + a_D N_D h_D} \tag{10}$$

Along the same lines, numerical intake rate R_D on *Dosinia* equals:

$$R_D = \frac{a_D N_D}{1 + p_L a_L N_L h_L + a_D N_D h_D}. \tag{11}$$

Substituting eq. 9 into eqs. 10 and 11, respectively, gives:

$$R_L = \frac{q}{s_L} \ , \tag{12}$$

$$R_D = \frac{a_D N_D (s_L - h_L q)}{s_L (1 + a_D N_D h_D)} \ . \tag{13}$$

These numerical intake rates are multiplied by the red knot feeding time per m^2 (expressed as V) for our Abelgh Eiznaya study area. The average red knot feeding density of 18.3 birds ha^{-1} was derived from unpublished observations of feeding red knots carried out in March 2007 and February 2008 over two and four days, respectively (J. A. van Gils, M. van der Geest & E. O. Folmer, *unpublished data*). Unfortunately, we have no detailed counts available during the period of sampling benthos (March 2011–February 2012), but the fact that

Table 6.1 Overview of the parameters used in the depletion model.

Parameter	Definition	Value	Unit	Source(s)
a_L	searching efficiency on *Loripes*	4	cm^2 s^{-1}	1, 2
a_D	searching efficiency on *Dosinia*	4	cm^2 s^{-1}	1, 2
c	max. digestive processing rate	5	mg DM$_{\text{shell}}$ s^{-1}	3
e_L	energy value *Loripes*	5.91	mg AFDM$_{\text{flesh}}$	4
e_D	energy value *Dosinia*	2.68	mg AFDM$_{\text{flesh}}$	4
h_L	handling time *Loripes*	1	s	1, 2
h_D	handling time *Dosinia*	1	s	1, 2
k_L	ballast mass *Loripes*	41.56	mg DM$_{\text{shell}}$	4
k_D	ballast mass *Dosinia*	34.31	mg DM$_{\text{shell}}$	4
q	max. tolerable toxin intake rate	0.1	mg AFDM$_{\text{flesh}}$ s^{-1}	5
s_L	toxin contents *Loripes*	e_L	mg AFDM$_{\text{flesh}}$	5
s_D	toxin contents *Dosinia*	0	mg AFDM$_{\text{flesh}}$	5

Sources: 1 J. de Fouw, T. van der Heide & J. A van Gils (*unpublished data*); 2 Piersma *et al.* (1995); 3 van Gils *et al.* (2003); 4 this study; 5 Oudman *et al.* (in revision).

we did not find a difference between the 2007 and the 2008 counts (Welch Two Sample *t*-test, $t = -0.34$, df = 3.11, $P = 0.75$), suggests that inter-annual variations in red knot numbers are negligible for our purpose. Nevertheless, we will explore the sensitivity of our results with respect to *V*, the more so because red knot densities at Abelgh Eiznaya tend to be higher than the average for Banc d’Arguin (Altenburg *et al.* 1982; van Gils *et al.* 2009). Assuming a daily feeding period of 14 hours (Altenburg *et al.* 1982; van Gils *et al.* 2005b; Leyrer *et al.* 2006) gives an estimated V of $1.07\ 10^{-3}$ feeding seconds per m^2 per second between mid-October and the end of May (i.e., 92.3 feeding seconds per m^2 per day). Since 1-year old red knots stay at Banc d’Arguin during summer (June–August), we assumed a predation pressure *V* that is only 7% of the value during October–May (van Dijk *et al.* 1990). The model predicts the depletion from mid-October to mid-August, using the densities observed in October as starting values, with available densities updated for depletion per step length Δt of 1 second in the following way for *Loripes* and *Dosinia*, respectively:

$$N_{L,t} = N_{L,t\text{-}1} - R_L V\,, \tag{14}$$

$$N_{D,t} = N_{D,t\text{-}1} - R_D V\,. \tag{15}$$

Next, *unavailable* densities are added to *available* densities in order to arrive at *total* densities at time *t* (where unavailable densities are the unavailable densities measured in starting month October, being 222.82 and 31.83 m^{-2} for *Loripes* (>4 cm depth) and *Dosinia* (depth >4 cm, length ≥13.2 mm), respectively).

Statistics

All statistics and graphs were carried out in R (R Development Core Team 2013), including the GAM models that were used to smooth seasonal trends in numerical densities (using package mgcv; Wood 2006). In these GAMs we added month as a non-parametrically smoothed function and sampling station as a parametrically estimated parameter.

Results

From 192 cores collected during the whole study period, a total of 3,763 mollusc specimens were retrieved. These comprised 20 species (Table 6.2) and represented eight orders and 19 families. Bivalves occurred in higher densities than gastropods, with nine bivalve species representing 88% of all specimens. Overall, the lucinid *Loripes lucinalis* dominated the assemblage (58% of all specimens), followed by the venerid *Dosinia isocardia* (10%) and the arcid *Senilia senilis* (8%).

The overall average biomass of the mollusc community amounted to 32 g AFDM m^{-2}, of which the West-African bloody cockle *Senilia* made up three-quarter (Fig. 6.2). Highest biomass densities were reached in February and July, and lowest in May (Fig. 6.2A). The contribution of *Senilia* was lowest in March–May, reaching a peak in the relative contribution of about 85% to overall biomass in February, June and July (Fig. 6.2B). On average, *Loripes* contributed 19%, a percentage that reached a minimum in July (9.6%) and a maximum in March (34.6%; Fig. 6.2B). Across the 20 species (Table 6.2), lowest densities were reached in late spring (May) and summer (Aug), whereas highest densities occurred in autumn (Oct).

Reflecting strong recruitment (as evidenced by the sudden occurrence of small, 2–6 mm long specimens in the samples), *Loripes* increased in density from 322 specimens m^{-2} in September to 785 specimens m^{-2} in October (Fig. 6.3A). Similarly, *Dosinia* increased from 18 specimens m^{-2} in August to 265 specimens m^{-2} in September (Fig. 6.3B), and *Senilia* increased from 50 specimens m^{-2} in July to 117 specimens m^{-2} in November (Fig. 6.3C). Two other bivalves that showed peak numbers in summer: *Modiolus* sp. (maximum of 149 specimens m^{-2} in July; Fig. 6.3D) and Diplodonta circularis, (maximum 95 specimens m^{-2}

Table 6.2 List of all mollusc species encountered (ordered alphabetically), with density and biomass during the month(s) with the lowest and highest numerical densities.

	Species lowest density month(s)			highest density month(s)		
	Month(s)	Density (m^{-2})	Biomass (g AFDM m^{-2})	Month(s)	Density (m^{-2})	Biomass (g AFDM m^{-2})
BIVALVIA						
Abra tenuis	5, 9–12	0.0	0.00	6	81.3	0.12
Diplodonta circularis	2	10.6	0.21	6	95.5	0.13
Dosinia isocardia	8	17.7	0.12	9	265.3	0.75
Loripes lucinalis	9	321.8	2.88	3	838.2	7.69
Modiolus sp.	3–4	0.0	0.00	7	148.5	0.55
Paphia dura	1–4, 6–12	0.00	0.00	5	7.1	0.59
Petricola pholadiformis	3	17.7	0.15	10	53.1	0.37
Senilia senilis	7	49.5	37.14	11	116.7	23.61
Tellina distorta	11	7.1	0.02	3	84.9	0.09
GASTROPODA						
Bittium reticulatum	2, 5–6, 8–11	0.0	0.00	12	212.2	0.70
Bulla adansoni	2, 6, 8	0.0	0.00	5	31.8	0.24
Clavatula bimarginata	1, 3, 5–7, 9	0.0	0.00	11	7.1	0.44
Columbella rustica	1, 3–12	0.0	0.00	2	3.5	0.01
Crepidula sp.	1, 3–11	0.0	0.00	12	3.5	0.02
Gibberula sp.	3–5, 7–8	0.0	0.00	10	35.4	0.07
Gibbula umbilicalis	4	0.0	0.00	10	159.2	1.94
Hydrobia ulvae	1–2, 4–5, 7–12	0.0	0.00	3	3.5	0.00
Mesalia mesal	3	0.0	0.00	11	70.7	1.10
Nassarius pfeifferi	1–6, 8–12	0.0	0.00	7	10.6	0.10
Prunum amygdalum	2, 4–6, 11	0.0	0.00	10	7.1	0.08
Total	8	675.5	25.57	10	1538.5	30.50

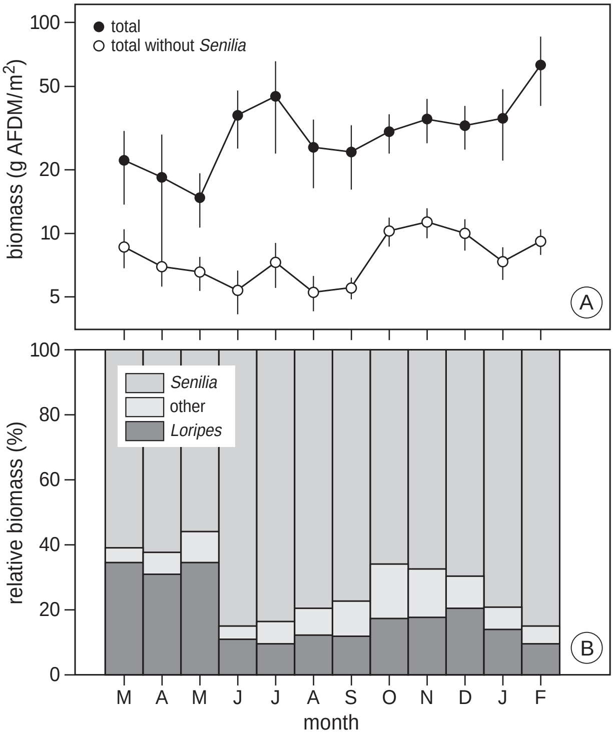

Figure 6.2 Seasonal changes in (A) total and total without Senilia biomass and in (B) community composition (in biomass terms) of molluscs at Abelgh Eiznaya, Banc d'Arguin, Mauritania, from March 2011 to February 2012.

in June; Fig. 6.3E), while *Tellina distorta* peaked in spring (maximum 85 specimens m^{-2} in March; Fig. 6.3F) and *Petricola pholadiformis* in autumn (maximum 53 specimens m^{-2} in October). The semelid *Abra tenuis* was found in several months but always in low densities that were too low to be able to detect clear seasonal variation. The venerid *Paphia dura* was encountered in May only.

Numerically, the gastropods represented 12% of the assemblage, with the trochid *Gibbula umbilicalis* (6%) being the only one common enough for us to detect seasonal fluctuations in their numbers (reaching its maximum density in October; Fig. 6.3G). The turritelid *Mesalia mesal* and the small snail *Bittium reticulatum* contributed 2% each, while the other gastropods contributed even less (<1% each; in decreasing order of abundance: *Gibberula* sp., *Bulla adansoni*, *Clavatula bimarginata*, *Prunum amygdalum*, *Nassarius pfeifferi*, *Columbella rustica*, *Crepidula* sp. and *Hydrobia ulvae*; Table 6.2).

The depletion model qualitatively matched the pattern of change observed in *Dosinia* and *Loripes* densities (Fig. 6.4). The initial fivefold decline in *Dosinia* densities (Oct–Mar), followed by a nearly twofold decline in *Loripes* density (Mar–Aug), strongly suggests that predation is the main driver behind these changes, especially predation by molluscivorous

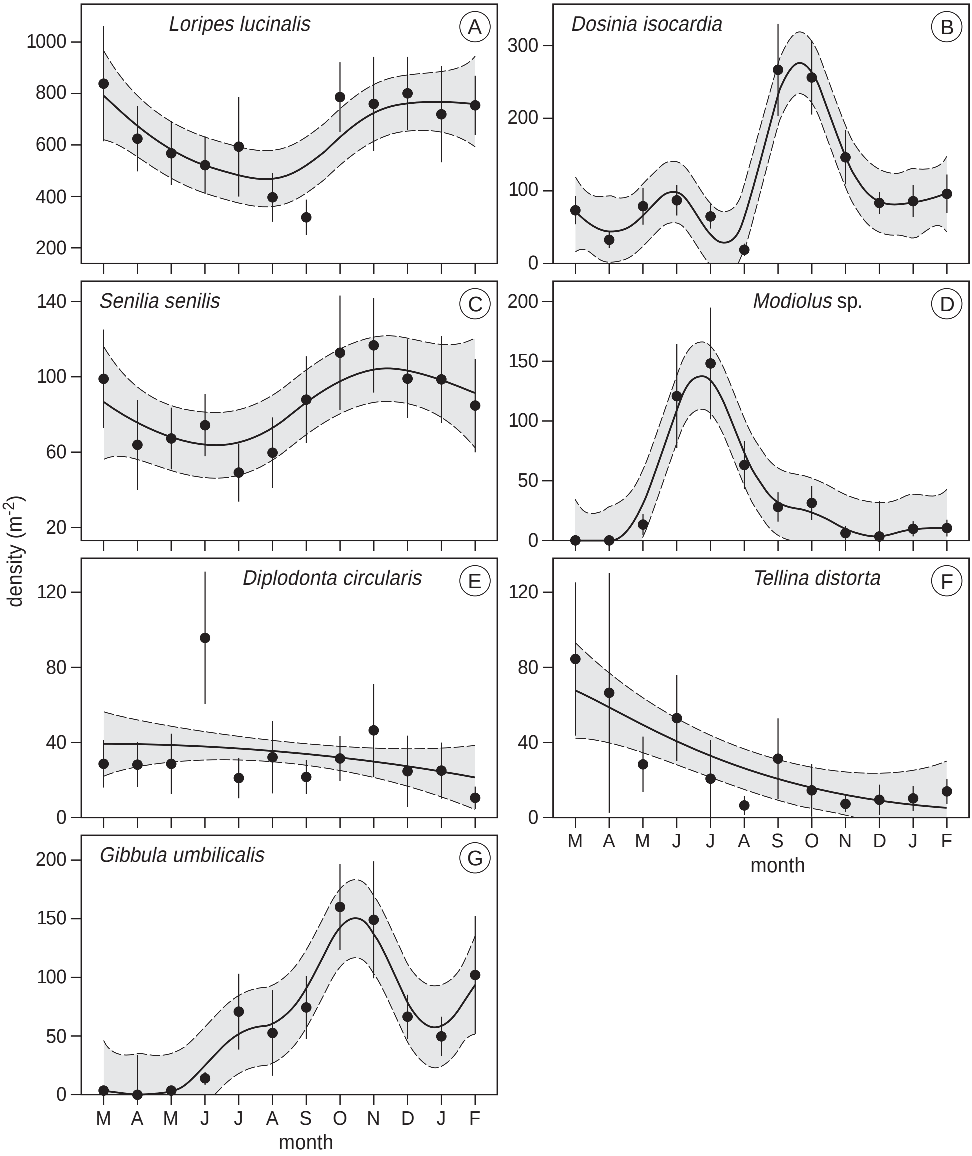

Figure 6.3 Seasonal changes in the densities of the seven most common mollusc species at Abelgh Eiznaya, Banc d'Arguin, Mauritania, from March 2011 to February 2012. Dots give means, bars give SE and smoothed lines give GAM fit (solid line) ± 2 SE (dashed lines).

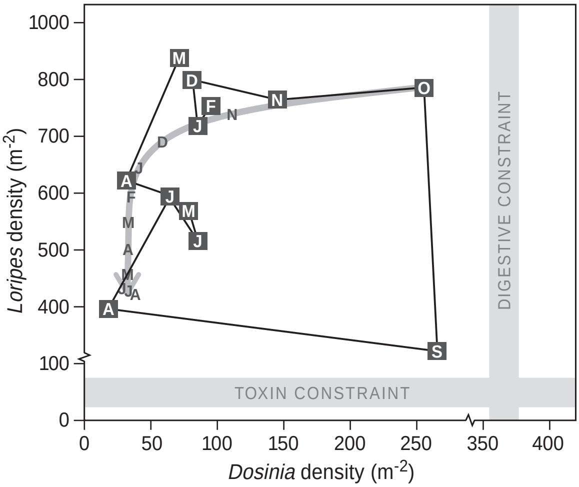

Figure 6.4 Biplot of the complementary density changes in the two bivalves *Loripes lucinalis* and *Dosinia isocardia* at Abelgh Eiznaya, Banc d'Arguin, Mauritania, from March 2011 to February 2012. Grey line gives the predicted depletion due to predation by red knots from mid-October to mid-August, taking October densities as starting values. Each filled dot on the grey line denotes a subsequent month in the simulation. Vertical and horizontal bars give critical densities above which molluscivorous red knots face a digestive and a toxin constraint, respectively. At these critical densities red knots achieve an intake rate that the birds can just sustain in the long run when it comes to digestive processing and tolerating the toxin load (for details how these critical densities are derived we refer to van Gils *et al.* 2013). Given the densities found in this study, red knots would only face their toxin constraint and not their digestive constraint.

red knots which should initially consume thicker-shelled *Dosinia* at a higher rate than the thinner-shelled but poisonous *Loripes*. In other words, the relative contribution of *Loripes* to the red knot's diet should gradually increase as *Dosinia* stocks become depleted, and is this indeed what the benthos data seem to suggest. Testing for the goodness of fit of the data (Oct–Aug) with the depletion model revealed that observed densities differ not from predicted densities, nor in *Loripes* ($\log_{10}$(observed density) = 0.61 + 0.81 × $\log_{10}$(predicted density), $R^2 = 0.65$, $F_{1,9} = 16.7$, $P = 0.003$; intercept does not differ from 0 ($P = 0.29$); slope does not differ from 1 ($P = 0.35$)), neither in *Dosinia* ($\log_{10}$(observed density) = 0.62 + 0.75 × $\log_{10}$(predicted density), $R^2 = 0.54$, $F_{1,9} = 10.5$, $P = 0.01$; intercept does not differ from 0 ($P = 0.15$); slope does not differ from 1 ($P = 0.30$)). These results remained unaffected when we carried out a sensitivity analysis by varying predation pressure V by ± 10% (V reduced by 10%: intercepts do not differ from 0 ($P = 0.70$ and 0.15 for *Loripes* and *Dosinia*, respectively), slopes do not differ from 1 ($P = 0.76$ and $P = 0.29$); V increased by 10%: intercepts do not differ from 0 ($P = 0.09$ and 0.15), slopes do not differ from 1 ($P = 0.12$ and $P = 0.32$)).

Discussion

In the present study, most (73%) of the mollusc biomass was contributed by *Senilia senilis*. Its relative share lies in the range of 63–85% found earlier for the entire intertidal Banc d'Arguin (Piersma 1982; Wolff *et al.* 1993a; selecting molluscs only in these studies). However, its absolute value (24.1 AFDM g m^{-2}) exceeds previous estimates that ranged from 4.7 to 8.1 g AFDM m^{-2} (Piersma 1982; Wolff *et al.* 1993a). Biomass densities of the other two abundant bivalves, *Loripes lucinalis* and *Dosinia isocardia*, are also higher than estimates made for the 1980s (5.2 vs. 2.6 g AFDM m^{-2} in *Loripes* and 0.3 vs. 0.1 g AFDM m^{-2} in *Dosinia*; Wolff *et al.* 1993a). However, note that sampling in the 1980s covered an area of about 30,000 ha, while our study area was restricted to about 50 ha only (Fig. 6.1). Hence, we cannot be conclusive about these differences.

Although numerous benthic studies have now been carried out on the Banc d'Arguin tidal flats (Piersma 1982; Wolff *et al.* 1987; Wolff *et al.* 1993a; Wolff *et al.* 1993b; Michaelis & Wolff 2001; Wolff & Montserrat 2005; Honkoop *et al.* 2008; Wolff & Michaelis 2008; van Gils *et al.* 2012), this is the first full-year survey of numerical changes in the molluscan assemblages in Banc d'Arguin. Such year-round studies on seasonality are rare anyhow (Beukema 1974; Wolff & de Wolf 1977; Dittmann 2002; de Goeij *et al.* 2003).

Mollusc biomass and numbers in our study showed considerable seasonal variation. Highest abundances occurred in the autumn and winter months and lowest in spring and summer, with a doubling of total abundance from August to October (Table 6.2). During the autumn months (September and October), the increase in abundance and species richness of molluscs has been generally related to recruitment events (Hodgson 2010; Chapter 2). This also seems the case in our study, as can for example be inferred from the fact that the doubling of total abundance from August to October is accompanied by an increase in total biomass of 19% only. The marginal biomass declines during summer (Jul–Sep; Fig. 6.1A) are most likely due to high temperatures leading to desiccation (Alongi 1990) and oxygen-limitation (Ferguson, White & Marshall 2013).

By contrast, the steep declines in densities, observed between autumn and spring, are most likely caused by intense predation by shorebirds spending their non-breeding season in large numbers at Banc d'Arguin (and in smaller numbers by young birds during summer; van Dijk *et al.* 1990). In fact, the observed reduction in biomass (36%; excluding *Senilia*; Fig. 6.2A) and numbers (45%; again excluding *Senilia*) between October and May parallels the calculations carried out on the basis of shorebird numbers and their energy budgets for tropical intertidal areas (p. 228 in van de Kam *et al.* 2004). Our findings differed to that of Wolff and Michaelis (2008), who did not find a difference in benthic biomass between autumn and spring. However, as admitted by these authors, their design did not allow discerning between an effect of season and an effect of year, since their autumn sampling was carried out in a different year (1988) than the spring sampling (1986). Furthermore, their comparison included three species of *available* bivalves only (it contained a fourth bivalve, *Senilia*, however that is largely unavailable to shorebirds because of its large size and thick shell; Wolff *et al.* 1987). Interestingly, among these three bivalve species, it was *Dosinia* that declined the steepest in their study (by 64%), a result that is in line with our depletion-by-red-knots model.

A final note is that predation takes place on individual prey and hence it may be better to compare numerical densities rather than biomass densities. As predation may lead to competitive release among prey, it may lead to higher *per capita* biomasses and/or growth rates among surviving prey (Gurevitch, Morrison & Hedges 2000). As a consequence, total biomass may even increase after predation (de Roos *et al.* 2007; Schröder, Persson & de Roos 2009). More to the point and being an example of release of *inter*specific competition, it has recently been shown that *Loripes* grows faster when *Dosinia* becomes depleted, presumably because *Loripes* is mixotrophic and also relies partly on the same resources as suspension-feeding *Dosinia* (van Gils *et al.* 2012; Chapter 2). Note that other predators than shorebirds, notably fish, may have also played a role in diminishing mollusc densities. However, although most fish species observed on the tidal flats at high tide feed on benthic fauna (Wolff *et al.* 2005), our two focal prey species, *Loripes* and *Dosinia*, do not seem to be important as prey species for fish (W. J. Wolff, pers. comm.). Shrimp, another potential predator of molluscs, are also commonly found at the Banc d'Arguin tidal flats (Schaffmeister, Hiddink & Wolff 2006). However, the size classes fed upon by shrimp are smaller than the size classes considered here (Andresen, Dorresteijn & van der Meer 2013). Most crabs that occur at Banc d'Arguin are deposit-feeding *Uca* sp. (Wolff *et al.* 1993a).

Taking a closer look at the most important avian molluscivore in our study system, the red knot, allows us to explain differences in the seasonal timing of depletion of several species of molluscs. Red knots have been accounted for being responsible for 80% of all predation on molluscs by vertebrates (van Gils *et al.* 2012), which justifies our focus on this predator of molluscan invertebrates. It has been shown elsewhere that the most important prey for red knots in Banc d'Arguin is *Dosinia* and not the much thinner shelled *Loripes* (van Gils *et al.* 2012; van Gils *et al.* 2013). Since red knots can only sustain an intake rate of 0.1 mg AFDM s^{-1} on diarrhoea-inducing *Loripes* (Oudman *et al.* in revision), the depletion rates on this species are relatively low (Fig. 6.4). Upon arrival from their breeding grounds, red knots in October have the luxury to consume *Dosinia* at a relatively high rate (because of a high *Dosinia* density) and add *Loripes* at a relatively low but constant rate of 0.1 mg AFDM s^{-1}. However, by the time that most available *Dosinia* have been depleted (and other small but less abundant non-toxic bivalves), in the second half of winter (Fig. 6.4), the relative contribution of *Loripes* to the red knots' diet increases (but still at the absolute rate of 0.1 mg AFDM s^{-1}) and peaks during spring/summer. This seasonal diet shift mimics differences in diet composition between years. In years that *Dosinia* is relatively poor, red knots include relatively much *Loripes* in their diet (65%), while in rich *Dosinia* years the proportion of *Loripes* in the diet levels out to around 20% (Onrust *et al.* 2013; van Gils *et al.* 2013).

This deterioration of feeding conditions for molluscivorous shorebirds may explain why fuelling rates of red knots at Banc d'Arguin are relatively low (Piersma *et al.* 2005). With an intake rate of at most 0.1 mg AFDM s^{-1} on *Loripes* only, red knots may have a hard time achieving an intake rate of at least 0.2 mg AFDM s^{-1}, which is the required level for fuelling at the Banc d’Arguin (van Gils *et al.* 2009). Thus, carrying capacity of tropical intertidal ecosystems for migratory shorebirds may be set by the spring food densities that remain after a long winter of intense predation by shorebirds exerted on molluscs.

Acknowledgements

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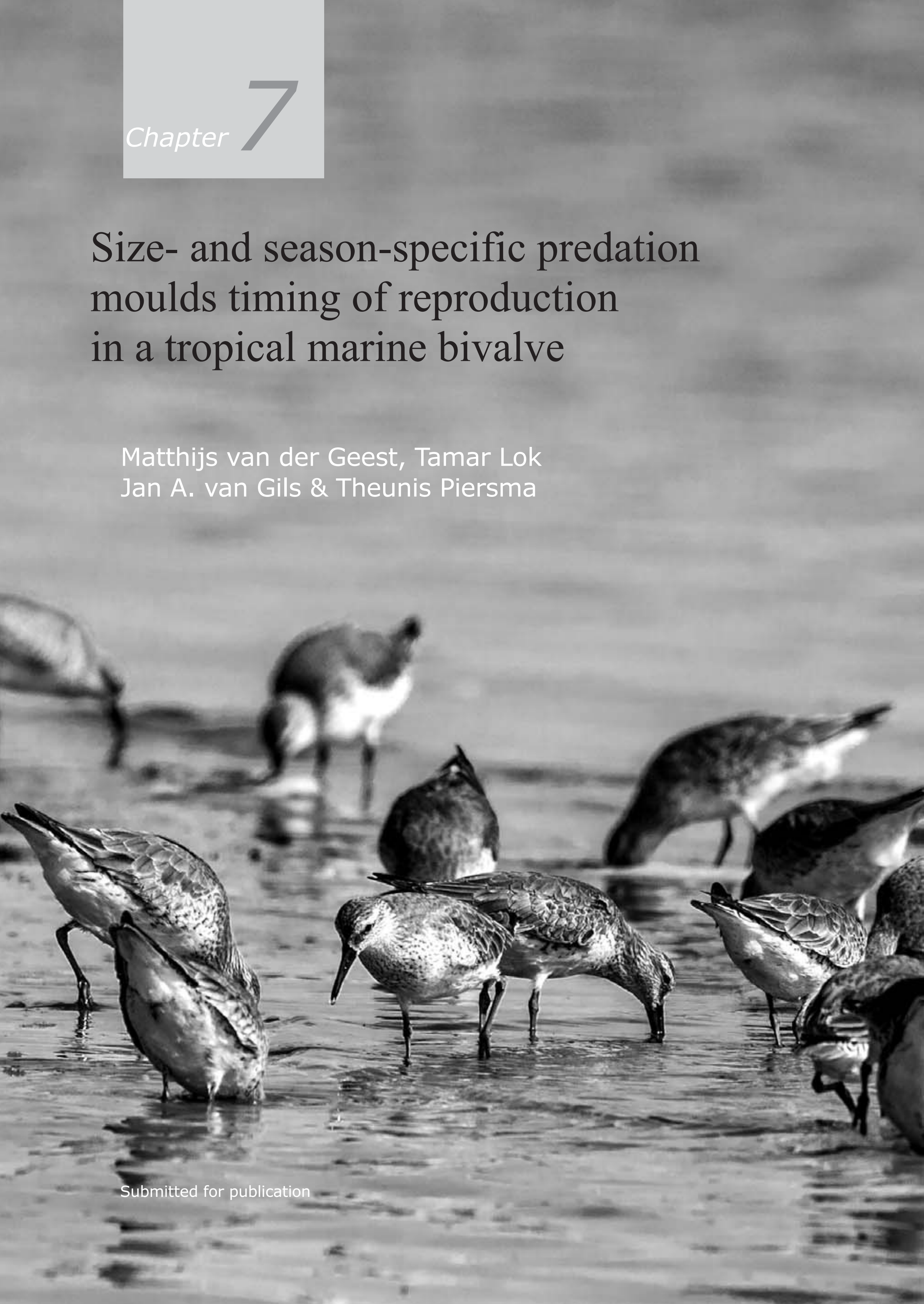

Chapter 7

Size- and season-specific predation moulds timing of reproduction in a tropical marine bivalve

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Submitted for publication

Abstract The reproductive cycle of the lucinid bivalve *Loripes lucinilis* in a tropical intertidal system (Banc d'Arguin, Mauritania) is characterized by two spawning periods: one in summer and one in late winter. A compelling feature of this reproductive cycle is that reproduction ceases during spring when resource availability and sediment temperature seem suitable to permit continued breeding. We here evaluate the potential selective importance of seasonality in pre-reproductive offspring mortality due to shorebird predation on timing of reproduction in *Loripes*. From September to April red knots (*Calidris canutus canutus*, i.e., shorebirds that spend the summer in the High Arctic breeding area) consume ~30% of the *Loripes* biomass. Based on *in situ* determined size- and season-dependent predation and growth rates, we quantified the date-of-birth-dependent offspring predation rate up to size at first reproduction. The reported spawning event in late winter coincides with relatively high offspring predation rates. This suggests that the benefits for *Loripes* to spawn prior to the spring growth season compensate for the relatively high offspring predation rate. However, that spawning ceased during the spring growth season when offspring predation rates were highest, while the second spawning event took place in summer when offspring predation rates dropped to relatively low levels, is consistent with the notion that size- and season-specific predation rates would mould timing of reproduction. That a combination of size- and season-specific predation may evolutionarily affect timing of reproduction in prey is a new inference, even though it may commonly occur.

Introduction

Earth is a seasonal planet, and this simple fact explains why organisms synchronize periods of reproductive activity with the times of the year that environmental conditions are best for growth and survival of offspring. Whereas the influence of seasonal fluctuations in food availability on the timing of reproduction is well documented for a variety of organisms (e.g., fish, Cushing 1990; birds, Daan, Dijkstra & Tinbergen 1990; spiders, Ward & Lubin 1993), the possible role of seasonal variation in predation risk has received less attention (but see Oksanen & Lundberg 1995; Naef-Daenzer, Widmer & Nuber 2001; Varpe *et al.* 2007). This is surprising, as predation, which is often size- or age-specific, is regarded a major selective factor when it comes to other life history traits such as age at maturity and size at birth (Kozlowski 1992; Wellborn 1994; Reznick *et al.* 1996; Day, Abrams & Chase 2002).

Because many predators are migratory and/or use different areas depending on the stage in their life cycle, seasonality in predation risk is a common feature of many ecosystems (Wilson 1991; Oksanen & Lundberg 1995; Kaartvedt 2000; Remmel, Tammaru & Magi 2009). As juveniles often experience higher risk of predation than adults due to their small size (Paine 1976; Chase 1999) and/or inexperience (Sullivan 1989; Tarwater *et al.* 2011), selection for timing of reproduction that minimizes predation risk during the immature life stage could be significant. This could be especially true in tropical systems where seasonality in food availability is assumed to be limited (Thorson 1950), providing opportunities for selective forces other than food to become important in moulding timing of reproduction.

In benthic marine invertebrates, high predation rates during pre-settlement life-stages are generally assumed to be significant in limiting recruitment success (Thorson 1950; Young & Chia 1987; Morgan 1995; Andresen, Dorresteijn & van der Meer 2013). Consequently, timing of reproduction as a function of predation on larval stages is considered to be an important selection pressure for individual fitness and thus for life history evolution in marine invertebrates (Morgan 1990; Morgan & Christy 1995; Hovel & Morgan 1997). The potential for post-settlement seasonal predation to affect the life histories (i.e., timing of reproduction) of marine invertebrates has received less attention, even though post-settlement seasonal predation can be severe. For example, in intertidal coastal areas, shorebirds (Charadrii) are known to have significant impacts on post-settlement benthic marine invertebrate prey populations (Zwarts & Drent 1981; Baird *et al.* 1985; Thrush *et al.* 1994; van Gils *et al.* 2012; Chapter 6). Many of these birds migrate from (sub-) tropical wintering grounds to breed in the high Arctic (Piersma & Wiersma 1996) during a very narrow time-window (Tulp & Schekkerman 2008). They also are size-selective predators due to gape-limitation (Zwarts & Blomert 1992). This leads to size- and season-specific predation that is predictable in duration and intensity (Wilson 1991). This makes tropical wintering migratory shorebirds and their benthic marine invertebrate prey a suitable model system to study post-settlement predation effects on reproductive strategies of their prey.

Densities of shorebirds wintering at the (sub)-tropical tidal flats of Banc d’Arguin, Mauritania (20ºN, 16ºW), are exceptionally high compared with other wintering areas along the East Atlantic Flyway (Zwarts 1988). The larger part of these tidal flats are covered with seagrass beds, in which the lucinid bivalve *Loripes lucinalis* (Lamarck, 1818; now considered synonymous with *L. lacteus*, Taylor 2012) is numerically the most abundant mollusk species (Honkoop *et al.* 2008; Chapter 6). Among the shorebirds wintering at Banc d'Arguin, the Afro-Siberian red knot (*Calidris canutus canutus*) is the most abundant molluscivore predator (Zwarts *et al.* 1998; Delany *et al.* 2009) for whom *Loripes lucinalis* (hereafter *Loripes*) forms an important food source (van Gils *et al.* 2013). Based on their densities, their diet composition, their minimum energy requirements and the reported standing stock of their prey we estimated that red knots should consume ~30% of the *Loripes* biomass over a single winter (see Appendix III, Section III.1).

In Chapter 2 we showed that *Loripes* reaches its size at first reproduction at ~7.5 mm in shell height and that the major spawning events occurred between January and February and between July and August. The interesting feature of this reproductive cycle is that reproduction ceases during the growth season (i.e., February to July), when food availability and sediment temperature seem suitable to permit continued breeding (see Chapter 2).

In search for an explanation we here explore the extent to which timing of reproduction in *Loripes* could be moulded by seasonal predation by size-selective red knots wintering at Banc d'Arguin. To do so, we used *in situ* collected data to determine size- and season-specific predation and growth rates of *Loripes* and examined whether the reported timing of spawning matched with the quantified optimal time of spawning that minimizes post-settlement offspring predation rate by red knots until size at first reproduction. To our knowledge this is the first study that provides empirical support that timing of reproduction is moulded by size- and season-specific predation.

Materials and methods

Prey size-distribution and biomass in the field

We collected 702 benthic samples in 4 consecutive expeditions: March–April 2007 ($N = 224$), January–February 2008 ($N = 142$), October–November 2009 ($N = 224$) and October 2010 ($N = 112$). All benthic samples were taken in the vicinity (< 5 km) of Iwik Peninsula, Banc d'Arguin, Mauritania (19º53' N, 16º18' W). A benthic sample constituted a sediment core (diameter, 15 cm), taken to a depth of 20 cm that was sieved over a 1-mm mesh. From each sieved benthic sample all *Loripes* individuals were sorted within 24 hours and preserved in 4% borax-buffered formalin. In the laboratory, shell length (L) of each living *Loripes* individual was determined (precision 0.1 mm), while shell height (H, precision 0.1 mm) and ash-free dry mass ($AFDM_{flesh}$) were determined of a subset of individuals. The latter was done by separating flesh from shell and drying it for 3 days at 60ºC. Next, dried flesh was weighed (precision 0.1 mg) and incinerated for 5 h at 550ºC; subsequently ash mass was determined (precision 0.1 mg). The $AFDM_{flesh}$ (in g)-to-length (L, in mm) relationships per expedition (March–April 2007, $AFDM_{flesh} = 10^{-3.96} L^{2.10}$, $N = 419$ specimens, $R^2 = 0.68$, $P < 0.001$; January–February 2008, $AFDM_{flesh} = 10^{-4.45} L^{2.45}$, $N = 82$

specimens, $R^2 = 0.90$, $P < 0.001$); October 2010, $\text{AFDM}_{\text{flesh}} = 10^{-4.75} L^{2.96}$, $N = 224$ specimens, $R^2 = 0.95$, $P < 0.001$) were used to predict $\text{AFDM}_{\text{flesh}}$ for the remaining individuals that were not incinerated, which enabled us to calculate the *Loripes* standing biomass (in g $\text{AFDM}_{\text{flesh}}$ m^{-2}) per expedition. Note that $\text{AFDM}_{\text{flesh}}$ was not determined for individuals collected in the October–November 2009 expedition. To predict *Loripes* standing biomass in this year, we used the October 2010 $\text{AFDM}_{\text{flesh}}$ -to-L relationship as we assumed this relationship to be mainly season-dependent and not year-dependent. This resulted in a *Loripes* biomass of 3.47, 4.01, 7.89 and 2.61 g $\text{AFDM}_{\text{flesh}}$ m^{-2}, respectively in March–April 2007, January–February 2008, October–November 2009 and October 2010.

Prey size-distribution in diet red knot

Red knots are molluscivore predators that ingest their hard-shelled prey whole, crushing them with their muscular gizzard (Piersma, Koolhaas & Dekinga 1993). The ingested shell material is excreted through the intestine and droppings can be used to reconstruct diet (Dekinga & Piersma 1993; Onrust *et al.* 2013). We collected a total of 33 dropping samples during 3 of the 4 expeditions to the Iwik Peninsula study area: March–April 2007 ($N = 7$), January–February 2008 ($N = 10$) and October–November 2009 ($N = 16$). Samples consisted of ~60 droppings on average. Samples were dried for three days at 60˚C; subsequently, *Loripes* shell fragments that were retained on a 300-µm sieve were sorted out. Next, height of all intact hinge + top fragments (H_{HT}, precision 0.1 mm) was determined as described by Onrust *et al.* (2013) to reconstruct ingested size-distributions per expedition. For this purpose we used the L (in mm)-to-H_{HT} (in mm) relationships determined per expedition (March–April 2007: $L = 1.058 + 8.695 H_{HT}$, $N = 154$ specimens, $R^2 = 0.80$; January–February 2008: $L = -0.5611 + 9.298 H_{HT}$, $N = 100$ specimens, $R^2 = 0.93$). Being analyzed by the same observer (i.e., Erik Rosendaal), the L-to-H_{HT} relationship obtained for March–April 2007 was also used to reconstruct the ingested *Loripes* size-distribution from hinges found in droppings collected in October–November 2009.

Size-specific daily predation rates

Having determined expedition-specific size-distributions of *Loripes* in the field and in the diet of red knots, the $\text{AFDM}_{\text{flesh}}$-to-$L$ relationship, the estimated standing stock of *Loripes* for each expedition, and the minimum *Loripes* biomass consumed by wintering red knots per day (2.16 mg AFDM m^{-2} day^{-1}; for calculations see Appendix, Section III.1), enabled us to empirically quantify the proportion of *Loripes* individuals eaten per size-class per day for the period in which wintering red knots are present in the study area (between September and April (equivalent to 241 days); Piersma 2007), the hereafter called daily predation rate per size-class (P_{day}). Van Dijk *et al.* (1990) estimated that 7% of the wintering red knot population at Banc d'Arguin stays over summer (between May and August). For this period (equivalent to 124 days) we therefore assumed a daily predation rate per size-class that is only 7% of the value between September and April.

Prey growth rate

We used the non-invasive technique of calcein staining to determine growth rates in *Loripes* (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 rate can then be determined as the maximum growth axis (in mm) 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) divided by the interval (in days) between calcein staining at t_1 and collection of the stained specimen at t_2. The technique was validated for burrowing *Loripes lucinalis* (van der Geest *et al.* 2011), to which we refer for a detailed description of the methods used. Briefly, during low tide, a PVC ring (diameter, 30 cm; height, 15 cm) was pushed 10 cm into the sediment. This 'basin' was then filled up by 0.5 L of calcein solution (containing 0.1 g calcein) after which the next high tide would flush the solution. PVC rings were removed next day. After a certain time interval, benthic samples were taken exactly at the spot where calcein was administered before, the sampling method being identical to that described above in the section *Prey size-distribution and biomass in the field*. From each sieved benthic sample all living calcein-treated *Loripes* specimen were sorted.

Despite the general believe that environmental conditions are more constant at lower latitudes (Thorson 1950), cyclic environmental events including fluctuations in temperature, precipitation, wind speeds and subsequent food availability are still present in tropical Banc d'Arguin (Chapter 2) and may result in seasonality in growth rates of *Loripes*. As this could influence the date-of-birth-dependent predation risk up to size at first reproduction, seasonal variation in growth rates should be taken into account. To detect seasonality in *Loripes* growth rate, growth rate was measured in different seasons of the year. As too long time intervals would dilute the potential presence of a seasonal effect on *Loripes* growth rate, the interval between time of calcein staining and sampling of the calcein-treated sites ($\Delta t = t_2 - t_1$) was restricted to a maximum of 200 days (~6.5 months). In addition, as too short intervals are likely to result in non-detectable growth, a minimum interval of 20 days was selected. Spread over the years 2007–2012, we randomly selected 71 sites distributed over the 7 tidal flats surrounding the Iwik peninsula at which we administered calcein *in situ*. At 57 of these sites, at least one *Loripes* was present and successfully marked. The average time interval between calcein marking and recapture at these 57 sites was 121 ± 59 (mean ± SD) days. Due to the restricted sample size, we were not able to account for potential differences in seasonal growth rates between years.

We fitted Von Bertalanffy's growth function (VBGF) to our data, a commonly used equation when modelling indeterminate bivalve growth. In this function, growth rate dH/dt declines with an increase in shell height H_t in the following way:

$$\frac{dH_t}{dt} = k\,(H_\infty - H_t) \qquad (1)$$

where H_∞ is the mean maximum size and k is the growth constant. To estimate the growth constant k from tag–recapture data, the traditional VBGF has to be modified using the derivation of Fabens (1965) increment model:

$$H_2 = H_\infty - (H_\infty - H_1)\,e^{-k\Delta t} \tag{2}$$

where k is the estimated growth constant and H_1 is defined as the shell height at time of marking (t_1), H_2 as the shell height at time of recapture (t_2), Δt as the time interval in days (i.e., t_2-t_1) and the mean maximum shell height H_∞ as 11 mm (consistent with van Gils *et al.* 2012; 99% of *Loripes* specimens collected in our study area between the years 2007 and 2010 were ≤11 mm in shell height ($N = 2{,}597$)). Rewriting this equation gives:

$$k = -\frac{\ln\left(\frac{H_\infty - H_2}{H_\infty - H_1}\right)}{\Delta t} \tag{3}$$

To deal with pseudoreplication (due to having multiple successfully marked *Loripes* individuals per benthic sample), we used the mean k per benthic sample for further analysis. The date (in days of the year running from 1 to 365) to which we allocated a given mean k was defined as the date exactly in between t_1 and t_2.

Quantifying date-of-birth-dependent pre-reproductive predation rates

Because growth was measured as an increase in shell height over time (see section *Prey growth rates* above), we used the regression coefficients of the L (in mm)-to-H (in mm) relationship ($H = -0.138+0.946L$, $N = 3{,}001$, $R^2 = 0.99$, $P < 0.001$) to express daily predation rate per shell height size-class (in mm).

Assuming that mortality is solely the result of predation by red knots, the survival rate per day of the year per size-class (S_{day}) can be calculated as 1-P_{day} for each size-class. Having determined the date of birth related growth function, we can estimate the number of days a *Loripes* individual would spend within each 1-mm size-class for each assigned date of birth. It is now possible to calculate the proportion of offspring lost to predation (P) for a given date of birth up to size at first reproduction, as follows:

$$\mathrm{P} = 1 - \prod_{t_1}^{t_2} S_{day(H,t)} \tag{4}$$

where t_1 is the assigned date of birth and t_2 is the date at which size at first reproduction (7.5 mm in shell height) is reached. We use the size-class and season-dependent daily survival rate in accordance with the date-of-birth-dependent time spent in each size-class to estimate P over time. We assumed size at birth (t_1) to be 0.2 mm (average size of a *Loripes* veliger is 0.17 mm; M. van der Geest, *unpublished data*) and H_∞ to be 11 mm (see section *Prey growth rate* above).

Statistical analysis

For the analysis of seasonal variation in *Loripes* growth rates (as represented by *k*), we used generalized additive modelling (GAMs; Hastie & Tibshirani 1990), as this enabled us to estimate fluctuations in *k* over time (in days, from 1 to 365) in a flexible way without having to choose a particular parametric form (Wood 2006). A Gaussian error distribution was chosen, which allows for negative values of *k*. Negative values of *k* might be the result of shell abrasion or measurement error. Smooth terms were represented using penalized regression splines (cyclic cubic regression splines with basis dimension $K = 7$), whose ends match, up to second derivative (Wood 2006). When we refitted the original model with an increased suspect *K* (*K* ranging between 7 and 15) there were no statistically important changes as a result of doing this, so $K = 7$ was considered large enough (results not shown). The optimum degree of smoothing for the smoother was defined by Generalized Cross Validation (GCV) as the scale parameter is unknown (Gaussian error distribution), increasing the amount that each model effective degree of freedom counts in the GCV score by a factor of $\gamma = 1.4$ (Wood 2006). GCV is known to have some tendency for occasional overfitting, and it has been suggested that using $\gamma = 1.4$ can largely correct this without compromising the model fit (Kim & Gu 2004; Wood 2006). Subsequently, the estimated smooth term for growth constant k over time was used to predict the date of birth related growth function which in turn was used to predict date-of-birth-dependent pre-reproductive predation rate. As there is some individual variation around H_∞ we performed a sensitivity analysis with respect to the value of H_∞. Daily predation rates per size-class obtained per expedition were logit-transformed before calculating the averages (Warton & Hui 2011).

All analyses were done in R (R Development Core Team 2013, version 3.0.0). For generalized additive modelling, the R-package 'mgcv' (Wood 2006) was used.

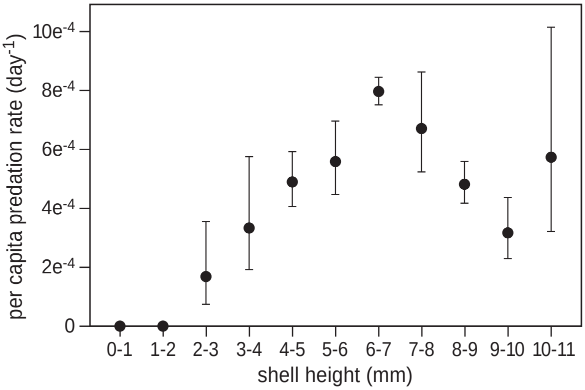

Figure 7.1 Per capita daily predation rate (mean ± SE based on values obtained in three consecutive years) of *Loripes lucinalis* as a function of shell height (mm) for the time of year (September to April) that all red knots (*Calidris c. canutus*) are present in the study area.

Results

Size-specific daily predation rate

Daily predation rate (P_{day}) by red knots between September and April was highly variable between size-classes increasing with size up to a maximum of $7.97 \cdot 10^{-4}$ for medium-sized *Loripes* (6–7 mm in shell height), after which P_{day} decreased for larger size-classes (Fig. 7.1). The observed sudden increase in P_{day} for the largest size-class (10–11 mm in shell height), together with the observed relatively large variation in P_{day} for this size-class seems to be a result of the limited number of *Loripes* reaching this size-class, making the prediction of P_{day} for this size-class very sensitive to occasionally ingested *Loripes* individuals belonging to this size-class. However, the highly variable P_{day} obtained for the 10–11 mm size-class will not affect our estimates of predation rate up to size at first reproduction, because adulthood is already reached at 7.5 mm in shell height (Chapter 2). There is some inter-annual variability in daily predation rate within each size-class, but the general pattern in daily predation rates across all size-classes is consistent for the 3 consecutive years (Fig. 7.1), which strengthened us in our believe that our approach to use the mean predation rate per size-class is valid.

Prey growth rates

The summary of our GAM to model growth constant k (day^{-1}) over time of the year is highly significant for the smoothed term ($F = 14.67$, $P < 0.001$, $N = 57$, $R^2 = 0.55$, GCV-score $= 2.237 \cdot 10^{-6}$): the effect of time in days on 4.96 estimated degrees of freedom. The model explained 59.2% of the deviance in k. Growth rates peaked between March and April, with a maximum predicted k-value in early April ($k = 0.0074$), and a second but smaller peak in predicted k in early July ($k = 0.0049$, Fig. 7.2). Furthermore, there was an extended period of low growth rates between September and January, with a minimum predicted k-value in mid-October ($k = 0.0013$, Fig. 7.2), while growth rates were also relatively low in May, with a minimum predicted k-value of 0.0025. Obviously, values for k will decrease when the assumed mean maximum value of shell height H_∞ is increased (see equation (2)). However, the fitted seasonal growth pattern was insensitive to the assumed mean maximum value of shell height H_∞ across a range of values for H_∞ (10–15 mm), reaching much beyond the natural range of H_∞ (10–12 mm; M. van der Geest and J. A. van Gils, *unpublished data*).

By using the fitted values of growth constant k (day^{-1}) over time (see Fig. 7.2), we could predict the pattern of shell growth for any given recruitment date up to size at 2 years (Fig. 7.3). By doing so, we see that the predicted shell growth is strongly influenced by time of recruitment: individuals recruited in spring (between 1 March and 1 May) have a relatively fast initial growth rate compared to individuals recruited at other times of the year. Furthermore, offspring born in spring spent the longest time in the size-class most predated by red knots (6–7 mm in shell height) at times when most red knots are present in the study area. In contrast, offspring born between 1 October and 1 December, escape severe predation by red knots while being in this 6–7 mm size-class (Fig. 7.3).

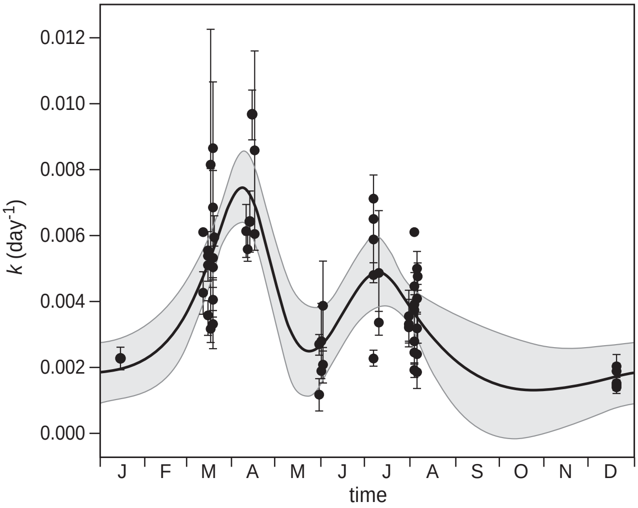

Figure 7.2 Estimated smooth term for the GAM model of growth constant *k* (day^{-1}) over time of the year (black line) for *Loripes lucinalis* and the corresponding 95% confidence interval (grey lines). Observed mean values for *k* are indicated by solid dots. Error bars show ± SE.

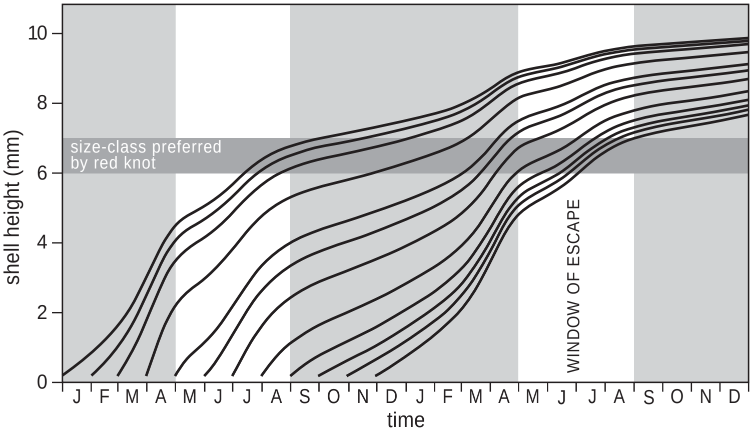

Figure 7.3 Predicted growth of *Loripes lucinalis* at Banc d'Arguin for 12 different recruitment dates (each recruitment date refers to the first day of each month of the year). White bars indicate windows of escape (i.e., periods that most molluscivore red knots (*Calidris c. canutus*) are absent). The dotted lines enclose the preferred *L. lucinalis* size-class eaten by red knots (see also Fig.7.1).

Date-of-birth-dependent predation rate up to adulthood

Size at first reproduction in *Loripes* was estimated to be reached at ~7.5 mm in shell height (Chapter 2). Plotting the proportion lost to predation (*P*) for a given date of birth until reaching size at first reproduction (i.e., adulthood) when accounting for seasonality in both

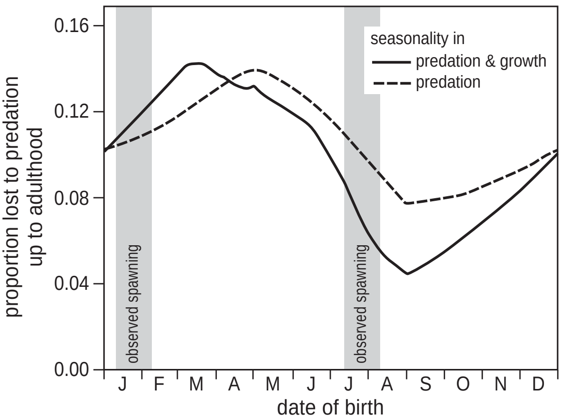

Figure 7.4 Proportion of *Loripes lucinalis* offspring lost to predation by red knots (*Calidris c. canutus*) up to adulthood (i.e., the probability of being predated before reaching shell height of 7.5 mm), for a given date of birth when including seasonality in (1) predation and growth rate (solid line), (2) predation rate only (dotted line). Grey bars indicate observed spawning periods (see Chapter 2).

predation and growth rates, revealed that *P* is greatly influenced by date of birth (Fig. 7.4; solid line). A maximum *P* was obtained when born in March, with *P* being 0.14, while a minimum *P* was obtained when born at the onset of September ($P = 0.04$). In other words, the chance to survive to adulthood for offspring born in early September is more than three times as high as the chance to survive to adulthood when born in March. This result was insensitive to using either the lower or higher 95% confidence interval estimates for growth constant k (day^{-1}) over time as presented in Fig. 7.2 (results not shown). The near absence of *Loripes* reproducing in between February and June (Chapter 2) coincides with a relatively high predicted predation rates for offspring born in this period, while the observed spawning events in July and January (Chapter 2), coincide respectively with relatively low and relatively high offspring predation rates up to adulthood (Fig. 7.4; solid line).

Discussion

In this study we empirically quantified seasonal patterns in date-of-birth-dependent pre-reproductive predation rates and assessed the match with the observed timing of reproduction in a tropical lucinid bivalve. That reproduction ceased during the growth season, when offspring pre-reproductive predation rate peaks (Fig. 7.4), strongly suggests that *Loripes* adjusts timing of breeding to enhance offspring survival to first reproduction. To our knowledge, this is the first study that has embraced ecological complexity by incorporating size- and season-specific predation and growth rates and size at first reproduction to empirically quantify offspring pre-reproductive predation rates as a function of date of birth. As survival until reproduction is one of the least understood components of demography

(Stearns 1992), we believe that integrating such complexity to estimate date-of-birth-dependent pre-reproductive predation is a necessity for a better understanding of population growth, life-history evolution and patterns of variation in life histories. However, increasing the amount of estimated parameters (with their associated errors) on which predictions are based may reduce robustness of these predictions. Therefore, we will first discuss the validity of our field-based data (i.e., time-dependent and size-specific predation and growth rates) on which we based our date-of-birth-dependent offspring pre-reproductive predation rate estimates.

Our observation that red knots are size-selective foragers with a preference for medium-sized *Loripes* (Fig. 7.1) is in agreement with observations on captive knots eating *Loripes* (Onrust *et al.* 2013) and with field observations by Zwarts and Blomert (1992) on knots feeding in the Dutch Wadden Sea on medium-sized *Macoma balthica* (Bivalvia). The reason that small *Loripes* are neglected is probably twofold: (1) small bivalves are harder to detect (Zwarts & Blomert 1992) and (2) require relatively long handling times which does not compensate for their higher digestive quality (van Gils *et al.* 2005b). However, whereas the avoidance of large *Macoma* could be explained by red knots being physically unable to ingest shells with a circumference of more than 30 mm due to gape-limitation (Zwarts & Blomert 1992), this argument cannot explain the negative selection of larger *Loripes*, because *Loripes* prey are all ingestible with their maximum circumference for $H_\infty = 11$ mm estimated at 26.4 mm. We believe that larger *Loripes* are negatively selected because they tend to be burrowed deeper in the mud (with a maximum burrowing depth of ~10 cm) making them inaccessible for red knots due to their limited bill length of ~3.5 cm. In addition, size-dependent digestive quality (i.e., the flesh-to-shell ratio) of prey may also play a role as digestive quality of *Loripes* decreases with size (M. van der Geest and J. A. van Gils, *unpublished data*).

For the summer period (May to August) when only 1-year old juvenile red knots stay in the study area, we assumed a daily predation rate per size-class that is only 7% of the size-specific daily predation rates observed in all the other months of the year (van Dijk *et al.* 1990). This value is somewhat arbitrary because breeding success of red knots is known to vary considerably from year to year (Boyd & Piersma 2001). However, a sensitivity analysis revealed that the seasonal differences in predicted date-of-birth-dependent predation lost up to adulthood were insensitive across a range of values for P_{day} between May and August (0–15% of the size-specific P_{day} values between September and April).

Growth rates were estimated to vary considerable throughout the year. Growth peaked in March and April and again (but to a lower extent) in June and July and was lowest between September and January (Fig. 7.2). This is consistent with the seasonal pattern in size-corrected body mass (BMI) that increased mainly from March to April and from June to July and decreased between September and January (Chapter 2). Using the same calcein marking technique and $H_\infty = 11$, van Gils *et al.* (2012) reported a growth constant k (year^{-1}) = 0.66 for *Loripes* from our study site. Correcting this value for the time interval between marking and recollection of the marked specimen (i.e., 1 year), using the derivation of Fabens (1965) increment model (see equation (1) and (2)), we obtain k (year^{-1}) = 1.08, which is equal to *k* (day^{-1}) = 0.00395. This *k*-value is very similar to the mean of the predicted *k* (day^{-1}) in this study (0.00308). The close similarity between our predicted *k* and that of van Gils *et al.* (2012) confirms that, despite the lack of growth data between September and November (Fig. 7.2), the reduced growth rates predicted for this period are likely correct.

In this study we aimed to explore how size- and season-specific predation may mediate timing of reproduction in *Loripes*. Since *Loripes* growth rates were highly seasonal (Fig. 7.2) we need to investigate to what extent the predicted seasonal pattern in date-of-birth-dependent pre-reproductive predation is driven by this seasonality in growth rate. Therefore, we did the same modelling exercise, but now assuming constant growth rates by using the daily growth rates averaged over the year (Fig. 7.4; dotted line). We found that the seasonal pattern in predicted date-of-birth-dependent pre-reproductive predation was already apparent when only assuming seasonality in predation pressure, but that the seasonal amplitude increased substantially when additionally accounting for seasonality in growth (Fig. 7.4). Ignoring seasonality in growth rates would thus have resulted in an underestimation of the potential selective force of seasonal and size-selective predation on timing of reproduction in *Loripes*.

High pre-reproductive predation rates for offspring born in spring may have selected for *Loripes* to cease reproduction during the spring growth season, while low pre-reproductive predation rates for offspring born in summer may have selected for *Loripes* to reproduce between July and August (Chapter 2). However, this cannot explain why *Loripes* also spawns between January and February (Chapter 2), as pre-reproductive predation rate is estimated to be relatively high for offspring born at that time (Fig. 7.4). Moreover, pre-reproductive predation rates were estimated to be minimal in early September, while *Loripes* reproduces about one month in advance between July and August. This suggests that other selective forces may also play a role in moulding the timing of reproduction in *Loripes*.

Food availability is generally considered another major selective force for timing of reproduction, with peak food demands of the offspring that should coincide with high food availability. Although we expected food availability to be relatively constant at our tropical intertidal study area, the observed seasonal variation in growth rate (Fig. 7.2) suggests it is not. Optimal growth conditions for the offspring may have been the selective force for reproduction between January and February, as growth rates increase rapidly in February to peak in April and May (Fig. 7.2). Pre-reproductive predation rate was lowest for offspring born in early September, but growth rates decreased rapidly between August and September. This may explain why *Loripes* reproduces slightly earlier between July and August, to take advantage of both relatively good growth conditions for the offspring and relatively low offspring pre-reproductive predation rates. In addition, being born prior to the growth season may increase offspring fecundity prospects, as, like many organisms, fecundity of bivalves is positively correlated with body size (Peterson 1986). Indeed, when we look at the size reached by the January and July cohort at the first and second future spawning opportunities (see Appendix, Fig. III.1), we see that while both cohorts reach the size of maturity (H = 7.5 mm) after exactly one year (i.e., the first spawning opportunity), the January-cohort has reached a larger size at their second spawning opportunity (9.4 mm), after 1.5 years, compared to the July-cohort (8.5 mm). This suggests that offspring born in winter reached higher fecundity compared to those born in summer. Thus, besides better offspring feeding prospects, higher offspring fecundity may explain why *Loripes* also reproduces in winter, when offspring predation risk is relatively high.

Although many studies discuss how size-specific predation or seasonal predation may act on life-history traits of prey, they have rarely empirically investigated the combined effect of size- and season-specific predation on these life-history traits. Contrary to the general believe that seasonality in pre-settlement offspring mortality risk regulates timing of reproduction in marine invertebrates, our work seems to be the first to provide empirical support that post-settlement size- and season-specific predation may also mould timing of reproduction in these organisms.

Acknowledgements

We are grateful to the Parc National du Banc d’Arguin (PNBA) for their permission to work in the park and for the use of their facilities. The PNBA crew at Iwik, and Lemhaba Ould Yarba at PNBA headquarters in Nouackchott helped in many different ways whereas, Joop van Eerbeek, Brecht de Meulenaer, Eelke Folmer, Erik Jansen, Han Olff, Jim de Fouw, Job ten Horn, Erik Rosendaal and Hanneke Gillis assisted in collecting and processing benthic samples. The latter two also analyzed dropping samples. Jaap van der Meer gave helpful comments on the analyses. This work was funded by the NWO-WOTRO Integrated Programme grant W.01.65.221.00 to T.P.

Appendix III

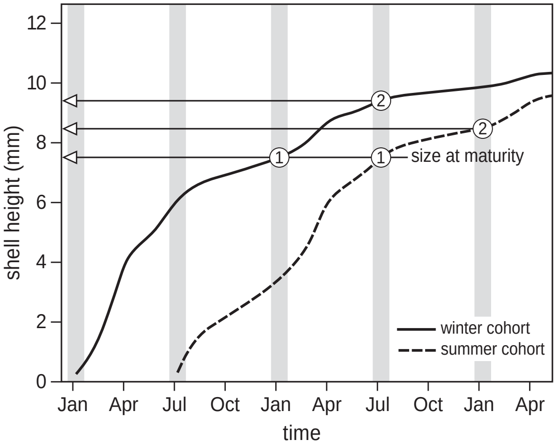

Figure III.1 Predicted growth pattern over time for the winter and summer cohort of *Loripes lucinalis*. Grey bars indicate observed spawning periods. Arrows indicate the shell height for both the winter and the summer cohort at their first (indicated by '1') and second (indicated by '2') life-time spawning opportunity.

Section III.1 Methods and results for estimating the daily and overwinter amount of *Loripes lucinalis* biomass consumed by wintering red knots (*Calidris c. canutus*) at Banc d'Arguin, Mauritania.

At Banc d'Arguin, wintering red knots (*Calidris c. canutus*) are present between September and April (equivalent to 241 days) each year (Piersma 2007), with an average density of ~5 individuals ha^{-1} (van Gils *et al*. 2009). Assuming a daily feeding period of 12 hours, it has been estimated that red knots feeding at Banc d'Arguin require a minimum intake rate of 0.2 mg ash-free dry mass (AFDM) per second to maintain a balanced energy budget (van Gils *et al* 2009). Shell-fragments in droppings collected at Banc d'Arguin in 3 consecutive years (2007–2009), reveal that the diet of red knots consists for ~50% of *Loripes lucinalis* (Onrust *et al* 2013) thus 4.32 g AFDM day^{-1} of *Loripes lucinalis* (hereafter *Loripes*) flesh is consumed by a single knot per day. This predation pressure (0.1 mg AFDM s^{-1} on *Loripes* only) is also expected and observed when taking the recently quantified maximum intake rate on *Loripes* into account (van Gils *et al* 2013), a maximum that is due to the mild toxic effects when red knots consume *Loripes* (Oudman *et al* in revision). Given the density of wintering red knots in our study area, we calculate that in total 0.521 g AFDM m^{-2} of *Loripes* is consumed by wintering red knots during their 241 day presence in the area, which is equal to a daily consumption of 2.16 mg AFDM m^{-2}. When averaging reported *Loripes* biomass estimations at Banc d'Arguin from published literature, the mean standing stock of *Loripes* at Banc d'Arguin is ~1.69 g AFDM m^{-2} (February–April 1986: 2.60 g AFDM m^{-2} (Wolff *et al* 1993a); September 1988: 1.06 g AFDM m^{-2} (Wolff & Michaelis 2008); December 2004: 1.42 g AFDM m^{-2} (Honkoop *et al* 2008: note that this value is obtained by correcting their stratified data for the 80% seagrass-coverage of the Banc d'Arguin intertidal flats). The estimated percentage of *Loripes* biomass predated by red knots over winter then becomes ~30%, although predation rates can be much higher at a local scale and for preferred size-classes. In light of this high percentage of post-settlement *Loripes* biomass being predated over the course of the winter season (September–April), we believe that our hypothesis of red knots moulding reproductive strategies of their lucinid bivalve prey is justified.

Chapter 8

Toxin constraint explains diet choice, survival and population dynamics in a molluscivore shorebird

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Abstract Recent insights suggest that predators should include (mildly) toxic prey when non-toxic food is scarce. However, the assumption that toxic prey is energetically as profitable as non-toxic prey misses the possibility that non-toxic prey have other ways to avoid being eaten, such as the formation of an indigestible armature. In that case, predators face a trade-off between avoiding toxins and minimizing indigestible ballast intake. Here we report on the trophic interactions between a shorebird (red knot, *Calidris canutus canutus*) and its two main bivalve prey, one being mildly toxic but easily digestible, and the other being non-toxic but harder to digest. A novel toxin-based optimal diet model is developed and tested against an existing one that ignores toxin constraints on the basis of data on prey abundance, diet choice, local survival and numbers of red knots at Banc d'Arguin (Mauritania) over 8 years. Observed diet and annual survival rates closely fit the predictions of the toxin-based model, with survival and population size being highest in years when the non-toxic prey is abundant. In the 6 out of 8 years when the non-toxic prey is not abundant enough to satisfy the energy requirements, red knots must rely on the toxic alternative.

Introduction

Toxic food is better avoided and there is a large literature on how predators learn to avoid toxic prey (Gittleman & Harvey 1980; Alatalo & Mappes 1996; Speed 2000; Endler & Mappes 2004; Greenlees, Phillips & Shine 2010; Halpin & Rowe 2010). Nevertheless, an emerging alternative view is that predators should not entirely neglect toxic prey as long as this could increase their opportunity to gain energy (Speed 1993; Sherratt 2003; Sherratt, Speed & Ruxton 2004; Barnett, Bateson & Rowe 2007; Skelhorn & Rowe 2007; Skelhorn & Rowe 2010; Barnett *et al.* 2012). Mildly toxic prey species that are not directly lethal upon ingestion could be valuable during times when non-toxic food is in short supply (Lindström *et al.* 2001; Kokko, Mappes & Lindström 2003). There are a number of cases where predators have been reported to consume toxic but not-immediately-lethal prey (Kicklighter, Fisher & Hay 2004; Gray, Kaiser & Green 2010; Beckmann & Shine 2011; Garcia, Sulkin & Lopez 2011; Lincango *et al.* 2011; Williams *et al.* 2011), but the dietary choices (Kicklighter, Fisher & Hay 2004; Gray, Kaiser & Green 2010; Skelhorn & Rowe 2010; Beckmann & Shine 2011; Lincango *et al.* 2011) and subsequent demographic consequences (Garcia, Sulkin & Lopez 2011) remain unexplained in mechanistic and functional terms.

Optimization models may help us to understand how predators should strategically trade off the minimization of toxin ingestion with the maximization of energy gain. Recent state-dependent models predict that the hungrier a predator, the more likely it is to accept toxic prey (Sherratt 2003; Sherratt, Speed & Ruxton 2004), a prediction that was upheld empirically (Barnett, Bateson & Rowe 2007; Barnett *et al.* 2012). Furthermore, through a predator's hunger state, the willingness to include mildly toxic prey should depend on the abundance and availability of non-toxic food, which is a prediction that allows field testing. However, when it comes to field testing, in both the models and the experiments, the only difference between prey types was their degree of toxicity, and this may be quite unrealistic.

In nature, prey species differ in many more defence traits than degrees of toxicity. By making it difficult for a predator to detect, capture, ingest or digest prey (Jeschke & Tollrian 2000; Caro 2005), non-toxic and nutritious prey species may escape predation. Predators therefore need to deal with multiple constraints, and may face much steeper trade-offs between energy gain and toxin avoidance than hitherto assumed. Here we will focus on such a system in which a predator faces the choice between an easy to digest toxic prey and a much harder to digest non-toxic prey. Building upon the existing digestive rate model (DRM) developed by Hirakawa (1995), which includes a digestive constraint but not a toxin constraint, we have developed a novel toxin-digestive rate model (TDRM) to generate food-density-dependent predictions on optimal diet and maximum energy intake rates for systems where prey differ in toxicity. The predictions of both the DRM and the TDRM are then put to the test in an 8-year field study on food abundance, diet choice, survival rate and population size in a molluscivore vertebrate predator, the red knot (*Calidris canutus canutus*, hereafter knot), in its non-breeding area at Banc d'Arguin (Mauritania), characterized by a highly sulphidic environment in which the most abundant mollusc prey is toxic, while other prey types are not.

Study system

The intertidal flats at Banc d'Arguin are densely covered by seagrass (mainly *Zostera noltii* Hornem.) (Wolff & Smit 1990). Detritus is produced at a high rate, which is degraded anaerobically by sulphate-reducing bacteria (Jørgensen 1982), causing a build-up of high concentrations of hydrogen sulphide (H_2S) in sediment pore-water (Calleja, Marbà & Duarte 2007; van Gils *et al.* 2012). Sulphide is toxic to many organisms as its lipid solubility enables it to freely penetrate biological membranes, eventually slowing down the functioning of mitochondria and the production of ATP (Bagarinao 1992). A specialized group of organisms that can profit from high sulphide concentrations in seagrass beds are Lucinidae (Taylor *et al.* 2011), heterodont bivalves that live in symbiosis with chemoautotrophic bacteria inside their gill structures (Taylor & Glover 2006). These bacteria oxidize sulphide that is provided by the lucinid host to synthesize sugars which fuel both the growth of the lucinid host and its endosymbiotic bacteria (Johnson, Diouris & Le Pennec 1994). The lucinid *Loripes lucinalis* (hereafter *Loripes*) is the dominant bivalve in Banc d'Arguin, with densities of up to 4,000 individuals per m^2 (van der Geest *et al.* 2011; van der Heide *et al.* 2012), and hence Banc d'Arguin can be considered as a chemosynthesis-based ecosystem (Dubilier, Bergin & Lott 2008).

Banc d'Arguin is an important nonbreeding area for Arctic-breeding shorebirds, hosting more than 2 million individuals in winter, with knots being the most abundant molluscivore (Altenburg *et al.* 1982). Knots face a trade-off between feeding on the superabundant but toxic *Loripes* (Oudman *et al.* in revision) and a much less abundant but non-toxic prey, *Dosinia isocardia* (hereafter *Dosinia*); numerically, *Loripes* and *Dosinia* together make up 75% of all molluscs that are ingestible by knots (Honkoop *et al.* 2008; Chapter 6) and dominate the diet of knots (Onrust *et al.* 2013). Knots face an additional trade-off: *Loripes* has a very thin shell, whereas *Dosinia* has a thicker armature. As knots ingest their prey whole (Piersma, Koolhaas & Dekinga 1993), they often face a digestive processing constraint (van Gils *et al.* 2003), which can be alleviated by selecting bivalves that have high flesh-to-shell mass ratios (van Gils *et al.* 2005b). The toxicity of *Loripes* for knots has recently been investigated experimentally (Oudman *et al.* in revision). Captive knots that were given a *Loripes*-only diet quickly developed diarrhoea, thereby losing significant amounts of water. Their compensatory water consumption could not prevent a decrease in food intake. When given a diet of non-toxic *Dosinia*, birds recovered within an hour. Intake rates on *Loripes* available ad libitum were three times lower than expected on the basis of maximal shell mass processing rates, whereas intake rates on *Dosinia* available ad libitum matched the prediction of a model that predicted intake as constrained by the processing of shells. When given the choice between *Dosinia* and *Loripes*, the captive birds included both prey types in their diet, which maximized their energy intake rate as predicted by a model developed for ad libitum situations.

Toxin-digestive rate model

The TDRM is developed for non-ad-libitum circumstances, where foragers need to search for their prey. In its most simple form, it assumes that there are just two prey types $i = 1,2$, which can each be characterized by energy contents e_i, indigestible ballast mass k_i, toxin contents s_i, handling time h_i, searching efficiency a_i and density D_i. The problem is finding the acceptance probabilities $\boldsymbol{P} = (p_1, p_2)$ for both prey types which maximize the forager's long-term energy intake rate Y. The latter is given by the multi-species version of Holling's disc equation (Holling 1959):

$$Y = \frac{p_1 a_1 D_1 e_1 + p_2 a_2 D_2 e_2}{1 + p_1 a_1 D_1 h_1 + p_2 a_2 D_2 h_2} \qquad (1)$$

In the 'classical prey model' (Stephens & Krebs 1986), which ignores possible digestive and toxin constraints, finding the optimal solution is straightforward. First, rank prey types such that $\frac{e_1}{h_1} > \frac{e_2}{h_2}$. Always accept type 1 ($p_1 = 1$), and accept type 2 ($p_2 = 1$) whenever $\frac{a_1 D_1 e_1}{(1+a_1 D_1 h_1} \leq \frac{e_2}{h_2}$, otherwise reject ($p_2 = 0$). This model, coined the 'contingency model' (CM) (Belovsky 1984), has been upheld in many diet studies on a variety of foragers (Sih & Christensen 2001), but was refuted in the case of knots (van Gils *et al.* 2005b; Quaintenne *et al.* 2010). As knots face a digestive constraint, they should and do take a prey's ballast mass into account when selecting their diet (van Gils *et al.* 2005b).

If ballast intake rate X for the optimal solution in the CM exceeds digestive constraint c, then the forager faces a digestive bottleneck, in which case the CM yields a suboptimal solution (Hirakawa 1995). Then, the rate-maximizing diet choice can be found using the digestive rate model (DRM) (Hirakawa 1995). This model can be solved graphically by plotting energy intake rate Y against ballast intake rate X for all possible combinations of $\boldsymbol{P}$, including partial preferences for either type (Fig. 8.1A). Then, by drawing digestive constraint c (vertical bar in Fig. 8.1A), one can work out which diet choice $\boldsymbol{P}$ yields the maximum sustainable energy intake rate Y under constraint c (asterisk in Fig. 8.1A). For details, we refer to the original paper by Hirakawa (1995) and its first applications in knots (van Gils *et al.* 2005b), for which such an 'all-or-nothing constraint' has explained intake rate (van Gils *et al.* 2003), prey choice (van Gils *et al.* 2005b; Quaintenne *et al.* 2010), patch choice (van Gils *et al.* 2005c), selection of stopover sites (van Gils *et al.* 2005a) and even digestive organ sizes (van Gils *et al.* 2003; van Gils *et al.* 2006; van Gils *et al.* 2007). As already mentioned by Hirakawa (1995), the same graphical procedure can be followed when the forager faces a toxin *rather than* a digestive constraint (replacing ballast intake rate X by toxin intake rate Z and ballast contents k_i by toxin contents si).

However, a forager's energy intake rate may be bottlenecked by *both* a digestive and toxin constraint. This occurs when, accounting for digestive constraint c in the DRM (i.e., when $X > c$ in the optimal CM solution), toxin intake rate Z in the optimal DRM solution exceeds q. This can only occur when the highest digestive quality prey (i.e., the one with the highest e_i/k_i) is most toxic (i.e., the one with the highest e_i/s_i; in our *Loripes–Dosinia* case, this condition was always upheld; see Appendix IV, Table IV.1). Graphically, the optimal solution under both constraints can be found by adding a third axis to Hirakawa's state space (Fig. 8.1B; note that we have added the third axis to the existing two-dimensional

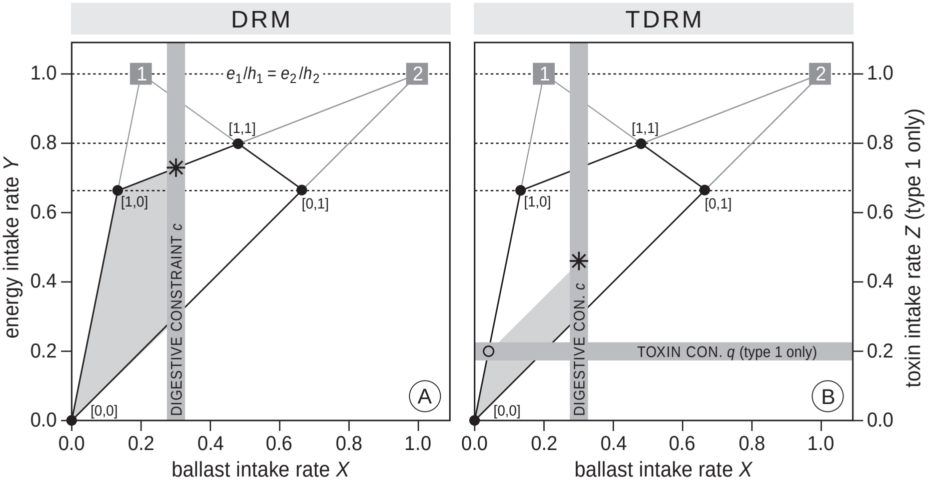

Figure 8.1 Graphical solution, following Hirakawa (1995) and van Gils *et al.* (2005b), to find the optimal choice between two prey types, which maximizes energy intake rate (asterisk in both graphs) under (A) one or (B) two constraints. In both graphs squared symbols give maximum intake rates at infinite densities of either type 1 or type 2 when there would be no constraints, kite-shaped surface in between the black solid lines gives feasible intake rates under (given) finite prey densities, grey area within the kite shape gives feasible intake rates under the acknowledgement of (A) a digestive constraint and (B) both a digestive and a toxin constraint. Numbers in squared brackets give diet choice as $[p_1, p_2]$. (A) Accounting only for a digestive constraint, the DRM ranks prey types on the basis of digestive quality (e/k) and predicts for this case that the high-quality prey (type 1) should be fully accepted ($p_1 = 1$), whereas the poor-quality prey (type 2) should only be partially selected ($0 < p_2 < 1$). (B) Accounting for both constraints, the TDRM predicts partial preference on both prey types ($0 < p_1 < 1$ and $0 < p_2 < 1$). Maximum energy intake rate is found by drawing a line parallel to the lower line of the kite shape (this line is parallel because toxin intake rate Z is kept at q across this line), starting where toxin constraint q crosses the left-most line of the kite shape (open dot) until it hits digestive constraint c (asterisk). Note that the scenario plotted here mimics our study qualitatively (the only toxic prey is the type with the highest e/k ratio), but not quantitatively (parameter values have been chosen arbitrarily).

plane, making reading the details easier; we could have also plotted X, Y and Z three-dimensionally). Solving the model analytically is equally straightforward and we will refer to it as TDRM (note that TDRM equals a DRM when only one of both constraints operates, which in turn equals a CM when none of the constraints is present). First, maximal sustainable ballast intake rate X is set by digestive constraint c,

$$X = c\ , \tag{2a}$$

which can be written as

$$\frac{p_1 a_1 D_1 k_1 + p_2 a_2 D_2 k_2}{1 + p_1 a_1 D_1 h_1 + p_2 a_2 D_2 h_2} = c\ . \tag{2b}$$

Similarly, maximally tolerable toxin intake rate Z is set by toxin constraint q,

$$Z = q \,, \tag{3a}$$

which can be written as

$$\frac{p_1 a_1 D_1 s_1 + p_2 a_2 D_2 s_2}{1 + p_1 a_1 D_1 h_1 + p_2 a_2 D_2 h_2} = q \,. \tag{3b}$$

Solving equations 2*b* and 3*b* for the two unknown variables p_1 and p_2 yields the optimal acceptance probabilities

$$p_1 = \frac{s_2 c - k_2 q}{a_1 D_1 (s_2 (k_1 - h_1 c) + s_1 (h_2 c - k_2) + q(h_1 k_2 - h_2 k_1))} \tag{4}$$

$$p_1 = \frac{k_1 q - s_1 c}{a_2 D_2 (s_2 (k_1 - h_1 c) + s_1 (h_2 c - k_2) + q(h_1 k_2 - h_2 k_1))} \,. \tag{5}$$

Materials and methods

Benthos

Our study period spans from 2003–2010, in which we collected 1,024 benthos samples in 13 consecutive expeditions: Dec. 2003 ($N = 84$), Dec. 2004 ($N = 26$), Apr. 2005 ($N = 39$), Dec. 2005 ($N = 8$), Nov. 2006 ($N = 6$), Apr. 2007 ($N = 229$), Aug. 2007 ($N = 8$), Oct. 2007 ($N = 12$), Feb. 2008 ($N = 142$), Apr. 2008 ($N = 78$), Nov. 2008 ($N = 56$), Oct. 2009 ($N = 224$) and Oct. 2010 ($N = 112$). Following procedures described elsewhere (van Gils *et al.* 2005b; van Gils *et al.* 2005c; van Gils *et al.* 2012), a benthos sample represented a sediment core (diameter: 15 cm) taken to a depth of 20 cm and sieved over a 1 mm sieve. Top (0–4 cm) and bottom (4–16 cm) part of the sample were sieved separately in order to distinguish between prey that are accessible and inaccessible to knots (Zwarts, Blomert & Wanink 1992). In the laboratory, each mollusc was identified to species level, and shell length was determined (± 0.1 mm). The latter allowed us to distinguish between ingestible and non-ingestible prey (knots can ingest all size classes of *Loripes* and *Dosinia* < 13.2 mm). By drying (3 days at 60ºC), weighing (± 0.1 mg), and incinerating (5 h at 550ºC) flesh and shell separately, we determined individual flesh ash-free dry mass $\text{AFDM}_{\text{flesh}}$ and shell dry mass DM_{shell} from subsamples. The relationships of $\text{AFDM}_{\text{flesh}}$ and DM_{shell} with shell length were used to predict missing values for those prey items that were not weighed. Next, numerical density (D in equations (1)–(5)), $\text{AFDM}_{\text{flesh}}$ (e in equation (1)) and DM_{shell} (k in equations (2b), (4), (5)) were averaged per year per species (available items only, i.e., those accessible and ingestible) and were used to calculate available biomass densities and as input variables in the two diet models (see Appendix IV, Table IV.1; toxin contents s was equated to flesh contents e in case of *Loripes* because toxin constraint q is expressed in terms of *Loripes* flesh intake). Further parameter values used were searching efficiency $a = 4$ cm^2 s^{-1} (Piersma *et al.* 1995; van Gils *et al.* 2012), handling time $h = 1$ s (van Gils *et al.* 2012), toxin constraint $q = 0.1$ mg AFDM$_{\text{flesh}}$ s^{-1} (Oudman *et al.* in revision; *Loripes* only), and gizzard mass = 10 g (van Gils *et al.* 2005a), resulting in digestive constraint $c = 5$ mg DM$_{\text{shell}}$ s^{-1} (van Gils *et al.* 2003).

All samples were taken in the vicinity (<5 km) of Iwik, Banc d'Arguin (19º53' N, 16º18' W). Samples collected in 2003, 2004 and 2006 were taken closer to Iwik (0–3 km) than in other years (1–5 km). Spatial differences at this scale might have had only little influence. Yet, smaller-scale spatial parameters such as distance to gullies, affecting the presence of seagrass (Folmer *et al.* 2012), might have had a larger effect. *Loripes* is mostly found in seagrass, whereas *Dosinia* is almost as abundant in bare as in seagrass habitat (Honkoop *et al.* 2008), and differences in prey densities between years may thus in part be due to differences in spatial design (on average, seagrass covers 80% of the intertidal surface at Banc d'Arguin (Wolff & Smit 1990)). We tested potential biases for both spatial scales by comparing our 2004 data (0–3 km to Iwik) with those of an independent study also from 2004 by Honkoop *et al.* (2008), who sampled mudflats 1–5 km away from Iwik and took an equal number of samples in bare and in seagrass habitat. 2004 was a notable year in which *Dosinia* was more abundant than *Loripes* (1142.7 versus 23.9 m^{-2} in our study and 216.6 versus 198.2 m^{-2} in the study by Honkoop *et al.* (2008); after correcting their stratified data for the 80% seagrass-coverage of the intertidal flats and for the species-specific availability fractions, 0.73 for *Dosinia* and 0.70 for *Loripes* (van Gils *et al.* 2012)). We repeated all analyses by replacing our 2004 benthos data by those of Honkoop *et al.* (2008), which revealed that neither the outcome of the survival analyses, nor the outcome of the diet comparisons were sensitive to our spatially inconsistent sampling program (see Appendix IV, Section IV.1).

Diet composition

During six of the 13 expeditions we collected 77 faecal samples (2003, $N = 21$; 2004, $N = 6$; Apr. 2007, $N = 8$; Oct. 2007, $N = 14$, Feb. 2008, $N = 11$; 2009, $N = 17$), samples usually containing 40–60 droppings. Samples were sorted using standard methodology (Dekinga & Piersma 1993), which has recently been calibrated for knots feeding on *Dosinia* and *Loripes* (Onrust *et al.* 2013). In short, after drying (3 days at 60ºC), shell fragments that were retained on a 300 µm sieve were sorted out and weighed per species, yielding species-specific estimates of ingested DM$_{\text{shell}}$ (after correcting for 35% of DM$_{\text{shell}}$ not being retained on the sieve (Onrust *et al.* 2013)). Next, hinges were assorted to species and their heights were determined in order to reconstruct ingested size distributions. The latter was needed to express a species's relative diet contribution in terms of total AFDM$_{\text{flesh}}$ consumed, because AFDM$_{\text{flesh}}$/DM$_{\text{shell}}$ ratios are size-dependent (Dekinga & Piersma 1993). Relative diet compositions were logit-transformed before calculating the annual averages (Warton & Hui 2011).

Annual survival rates

Survival estimates were based on capture/resighting data of a total of 1595 individually marked knots. The birds were captured and resighted during annual three-week expeditions in November/December 2002–2010 (Leyrer *et al.* 2012), yielding annual survival estimates for seven consecutive years (2003–2009; because survival rate cannot be separated from resighting probability for 2010 when modelled with time dependence). The birds were aged upon capture (Prater, Merchant & Vuorinen 1977), distinguishing hatch-year birds (juveniles) from older birds (adults). Apparent (or local) survival (Φ) and recapture probabilities (p) were estimated from live encounter data using Cormack-Jolly-Seber models (Lebreton *et al.* 1992). As benthos and diet data were collected throughout the entire study area, we pooled the data of the two sites in our study area, Abelgh Eiznaya and Baie d'Aouatif (Leyrer *et al.* 2006; Leyrer *et al.* 2012). Based on knowledge gained from earlier analyses, we made some *a priori* assumptions to reduce the number of parameters in order to increase the precision of the survival estimates: it has been shown that a time-since-marking (tsm) effect explained most of the variation in annual survival (Leyrer *et al.* 2012), and we thus considered tsm-effects to account for transients or handling effects on survival in the first year after capture (Φ^1) versus subsequent years (Φ^{2+}). It has further been shown that age at capture (adult versus juveniles) explained a significant part of the variation in survival (Leyrer *et al.* 2012), and we thus included age at capture in our models. Note that knots were treated as adults after their first year (more than 12 months of age), and consequently no age differences existed within the Φ^{2+} category. As we were interested in which of the two diet models best explained the annual variation in survival rate, we included intake rates predicted by the TDRM and DRM, respectively, as continuous variables in the models. Additionally, to test for survival differences among years, we included time as a factor (time), but also tested whether there was a linear trend in survival rate over time (Time), because an earlier analysis indicated a decline in knot survival over time (Leyrer *et al.* 2013). In all models, resighting probability p was modelled as a function of time (again as a factor) and site, as observation effort differed between the two sites, and logistic improvements suggested resighting efforts differed between years (Leyrer *et al.* 2012). Both adults and juveniles forage on open mudflats during low tide and assemble at roosts during high tide and we had no reason to expect p to differ between age classes.

The global model was $\Phi_{\text{age*tsm+time}}\, p_{\text{site+time}}$ and we tested the goodness of fit using the median-$\hat{c}$ (c-hat) test implemented in the MARK software (version 6.0; White & Burnham 1999). The level of overdispersion was estimated at $\hat{c} = 1.05 \pm 0.00$. Models were constructed and run in R (version 2.15.0) using the RMark version 2.1.4 package (Laake 2012) as an interface for program MARK (White & Burnham 1999). We used model averaging to calculate survival and resighting probability, and present parameter estimates as $\hat{\theta} \pm 1$ SE. Model selection was based on Akaike's Information Criterion corrected for small sample size and overdispersion ($\hat{c}$; QAIC$_c$). Based on the earlier-mentioned assumptions, the candidate model set consisted of all biologically and ecologically plausible combinations of parameterizations for Φ and p (see Appendix IV, Table IV.2).

Estimating and predicting population dynamics

Each year between 2002 and 2010 we carried out a single count of all knots roosting in the Iwik study region. This took place during a daytime spring high tide in November/December. Birds were counted using telescopes by four or five teams of two observers, each counting a subsection of our study area.

We modelled the population trend for 2002–2010 using adult and juvenile survival rates estimated by the most parsimonious model (i.e., survival model 1 in Appendix, Table IV.2). In this statistical model, TDRM energy intake rates Y served as input, which were predicted on the basis of equation 1 using (i) the observed densities of both *Loripes* and *Dosinia*, (ii) the observed densities of *Loripes* only, and (iii) the observed densities of *Dosinia* only. The last two hypothetical scenarios allow us to hypothesize how much knot population dynamics depend on the presence of either *Loripes* or *Dosinia*. As applied before when modelling knot population dynamics (Baker *et al.* 2004), we used a two-dimensional matrix population model, in which fecundity (f; equal to 0 for juveniles and 0.14 yr^{-1} for adults (van den Hout *et al.* in revision)), juvenile survival (Φ_{juv}) and adult survival (Φ_{ad}) determine how the number of juveniles (N_{juv}) and adults (N_{ad}) in year t affect the number of juveniles and adults in year $t + 1$:

$$\begin{bmatrix} N_{\text{juv}} \\ N_{\text{ad}} \end{bmatrix}_{t+1} = \begin{bmatrix} 0 & f \\ \Phi_{\text{juv}} & \Phi_{\text{ad}} \end{bmatrix}_t \begin{bmatrix} N_{\text{juv}} \\ N_{\text{ad}} \end{bmatrix}_t \qquad (6)$$

The 2002 count was used as the initial population size in the model.

Results

Annual survival rate

TDRM models were substantially better supported than models including DRM intake rates (cumulative QAIC_c weight: 0.38 for models including TDRM intake rates, and 0.00 for models including DRM intake rates; Appendix, Table IV.2; Figs 8.2A–B). Although models including annual variation as explanatory factor (i.e., factor time) scored high in the model selection process (cumulative QAIC_c weight: 0.48; Appendix, Table IV.2), they added extra parameters (complexity) to the models and should thus be less favoured. There was no evidence for a time trend in survival (i.e., models including Time; cumulative QAIC_c weight: 0.14; Appendix, Table IV.2). Furthermore, there was no support for adult survival being different in the first year after marking, compared to subsequent years (model 2 versus model 3, $\Delta\text{QAIC}_\text{c} = 0.34$). Model averaged survival estimates can be found in the Appendix, Table IV.3.

Diet composition

The observed contribution of *Loripes* to the diet was less than predicted by the DRM (Figs. 8.2C; $t = -3.44$, df = 4, $P = 0.03$). For 3 out of 5 years for which we had diet data available, the DRM predicted that knots should fully ignore *Dosinia* (Fig. 8.2C). In those three years

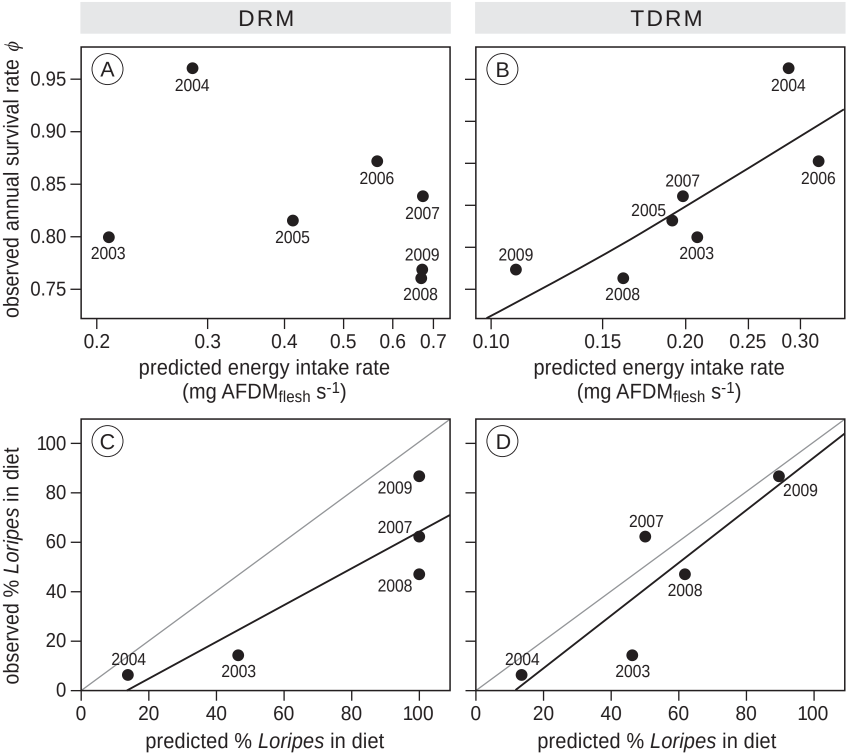

Figure 8.2 (A) Year-specific adult survival rate (estimated by model 2 in Appendix, Table IV.2; year runs from Nov/Dec of the previous year to Nov/Dec of the plotted year) does not correlate with the DRM-predicted intake rate, (B) whereas it correlates positively with the intake rate predicted by the TDRM. Line gives model fit (model 1 in Appendix, Table IV.2). (C) Observed amounts of *Loripes* in the diet (relative to *Dosinia*) are lower than predicted by the DRM, (D) but match with TDRM-predictions. Grey lines represent $y = x$ lines, and black lines are significant regression lines.

(2007, 2008, 2009), the abundance of *Loripes* was so high that, even if knots would feed on *Loripes* only –the prey with the highest flesh-to-shell mass ratio–, their gizzard would not be able to achieve the required shell mass processing rate (i.e., knots would face a digestive constraint). Hence, only a proportion of encountered *Loripes* should have been accepted (see Appendix IV, Table IV.4; note that this is different from conceptual Fig. 8.1A where, for reasons of visual clarity, we assumed that even maximum ballast intake rates on prey type 1 (i.e., k_1/h_1) are below digestive constraint c).

By contrast, diet compositions predicted by TDRM matched the observed diets (Fig. 8.2D; $t = -1.26$, df = 4, $P = 0.28$). In 3 out of 5 years the intake rate on *Loripes* would have exceeded the toxin constraint if all encountered *Loripes* were accepted. Hence, only a proportion of the encountered *Loripes* should have been accepted for this reason (see Appendix IV, Table IV.4). In those years, knots following the TDRM could accept all encountered (ingestible) *Dosinia* as the occurrence of the toxin constraint kept shell mass

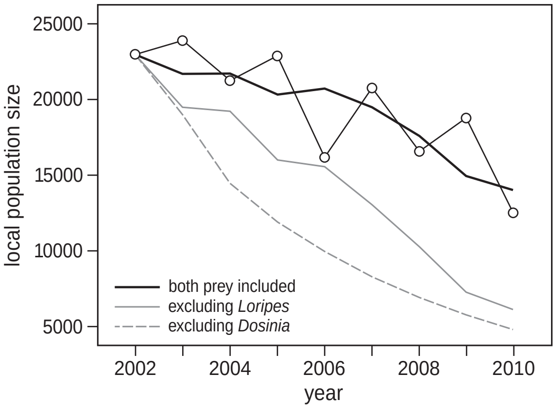

Figure 8.3 Predicted population dynamics of knots in the presence of both prey (thick black line), in the absence of *Loripes* (solid grey line), and in the absence of *Dosinia* (dashed grey line). Observed population size (circles connected by thin black line) follows predicted population decline based on both prey.

processing rates low, and thereby prevented a digestive constraint. Only in the year that *Loripes* was less abundant than *Dosinia* (2004) does the TDRM predict a digestive rather than a toxin constraint. In 2004, knots should thus have accepted all encountered *Loripes* and only a fraction of the encountered (ingestible) *Dosinia* (see Appendix IV, Table IV.4).

Predicted and observed population dynamics

Predicted knot population size declined over time, with the decline being steepest if *Dosinia* would have been removed from the system (-79% from 2002 to 2010), followed by the scenario when *Loripes* would have been removed (-74%). However, even with both prey included in the diet, knot numbers were predicted to decrease over time (-39%; Fig. 8.3). This last model agreed best with the observed decline in knot numbers from 22,859 in 2002 to 12,465 in 2010 (-45%; Fig. 8.3).

Discussion

Knot annual survival rates correlated strongly with annual variations in *Dosinia* abundance (Fig. 8.4A; Pearson's $r = 0.91$), but showed no trend with *Loripes* abundance (Fig. 8.4B; Pearson's $r = –0.72$). This strongly suggests that knots need non-toxic *Dosinia* to survive and cannot rely on *Loripes* only, even though *Loripes* is much more abundant and has a much higher flesh-to-shell ratio. The reasoning for this dependency is rather simple: in order to prevent lethal intoxication, knots can ingest *Loripes* up to a rate that is only half of their required intake rate (Oudman *et al.* in revision), and they need prey such as *Dosinia* to meet their energy demands. On the other hand, *Dosinia* was not abundant enough for knots to fully rely on them as an energy source.

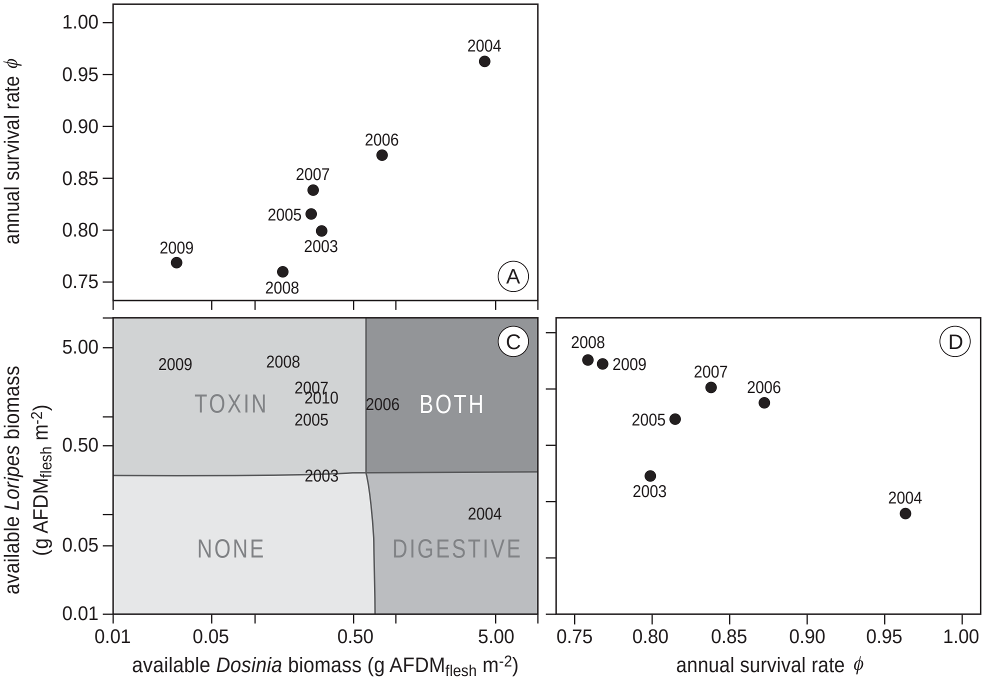

Figure 8.4 (A) Year-specific adult survival rate (estimated by model 2 in Appendix, Table IV.2) correlates with the available biomass density of *Dosinia*, (B) but not with *Loripes* density. (C) These prey densities themselves correlate negatively. Grey shading in the background indicates whether knots would either face a toxin constraint, a digestive constraint, both constraints or neither. For details behind these calculations see Appendix, Section IV.2 and Fig. IV.1.

The TDRM, which seems to capture the essence of the knots' dietary problem, assumes the following strategy: accept toxic but energy-rich *Loripes* until toxin constraint is met, then add bulky *Dosinia* until the digestive constraint is met. According to our calculations, knots faced both constraints only in 2006 when both prey species occurred in high densities (Fig. 8.4C; for details calculations see Appendix IV, Section IV.2 and Fig. IV.1). In most years (six of eight; Fig. 8.4C) however, *Dosinia* was not abundant enough for the birds to become digestively constrained, whereas the presence of *Loripes* was usually high enough to meet the toxin constraint (Fig. 8.4C). This explains the negative correlation between the *relative* amount of *Loripes* in the diet and the available density of *Dosinia* (Fig. 8.5A): although the *absolute* rate at which *Loripes* was eaten was likely to be constant each year (equal to toxin constraint q), the *absolute* rate at which *Dosinia* was eaten increased with the available *Dosinia* density as long as birds were not digestively constrained (this would occur at a *Dosinia* density of 0.6–0.7 g $\text{AFDM}_{\text{flesh}}$ m^{-2}). A recent study showing year-round changes in *Dosinia* and *Loripes* densities also suggests that the relative contribution of *Loripes* to the diet of knots increased as *Dosinia* stocks became depleted throughout winter (see Chapter 6).

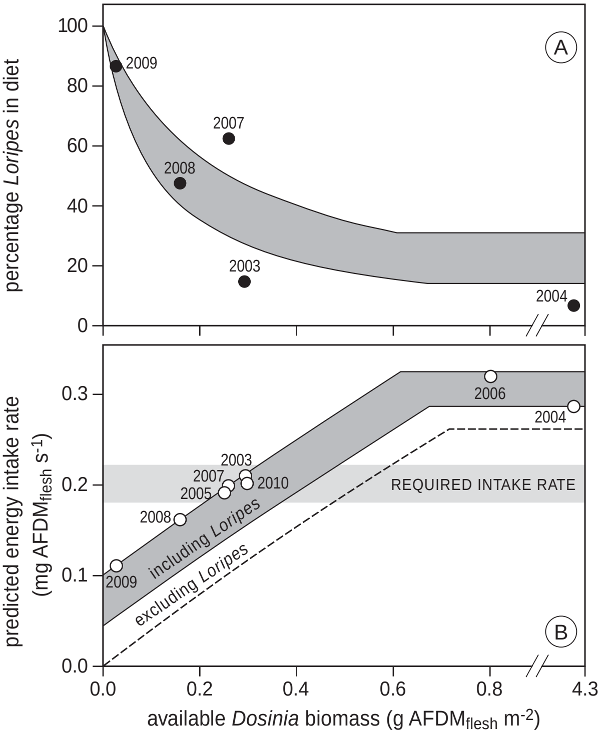

Figure 8.5 (A) How the amount of *Loripes* in the diet (relative to *Dosinia*) relates to the available density of *Dosinia*, both theoretically (TDRM) and empirically. Theoretical predictions are given by the grey band, with lower line representing a poor *Loripes* density (0.1 g AFDM m^{-2}) and upper line a higher *Loripes* density ($\geq$ 0.25 g AFDM m^{-2}; as knots face a toxin constraint at *Loripes* densities of at least 0.25 g m^{-2}, diet composition becomes independent of *Loripes* abundance above such densities). Diet composition becomes independent of *Dosinia* density when the digestive constraint is met (i.e., above *Dosinia* densities of 0.6–0.7 g m^{-2}). (B) TDRM functional response to variations in *Dosinia* density. Grey band as in (A) shows that most variation in intake rate is due to density variations in *Dosinia* rather than in *Loripes*. Nevertheless, without *Loripes*, intake rates would be substantially lower (dashed line) and often below the level required for subsistence. Dots denote year-specific predictions based on *Loripes* and *Dosinia* densities.

In Banc d'Arguin, knots need an average energy intake rate of c. 0.2 mg AFDM$_{flesh}$ s^{-1} in order to maintain body mass (van Gils *et al.* 2009). In most years, knots would only achieve half of this rate if they would fully neglect *Loripes* and only accept *Dosinia* as their prey. By adding *Loripes* to their diet knots would just meet their required energy demand. A plot of the predicted intake rate with (grey band in Fig. 8.5B) and without (dashed line in Fig. 8.5B) *Loripes* against the available *Dosinia* densities shows that energy intake rate without accepting *Loripes* would be insufficient for subsistence in 6 of 8 years (also see Appendix IV, Table IV.4). Only in 2004 and 2006 would knots have been able to achieve their minimum energetic requirements on *Dosinia* alone (see Appendix IV, Table IV.4; although we modelled knots as 'intake rate maximizers', they could just as well have featured as 'sulphide minimizers' in these 2 years by fully ignoring *Loripes*; however the diet data available for 2004 suggest they did not; Fig. 8.5A).

Note that rate maximization *while* feeding allows for the minimization of daily feeding time if a fixed amount of *daily* energy is required (Schoener 1971). Minimizing daily feeding time can be beneficial if foraging comes at a cost, such as for example enhanced predation risk (McNamara & Houston 1994). This justifies our approach to analyse survival as a continuous function of intake rate rather than as a simple step function of whether metabolic demands are met. Note further that in poor *Dosinia* years, notably in 2009 (see Fig. 8.5B; Appendix, Table IV.4), knots would not even have been able to survive on the combination of *Loripes* and *Dosinia* alone and would have needed to include other prey types in their diet (which knots indeed did, especially in 2009 (Onrust *et al.* 2013)).

With *Loripes* and *Dosinia* being by far the most abundant available bivalves at Banc d'Arguin (Honkoop *et al.* 2008), there are not many alternative mollusc prey to include in the diet. This notion, and the fact that the last years of our study period have not shown high densities of *Dosinia* (Fig. 8.4C), may explain why the local knot population has declined during especially the second half of our study period (Fig. 8.3). However, TDRM energy intake rate showed no trend over time ($r = 0.51$; $F_{1,6} = 2.07$; $P = 0.20$). In addition, also in 1980s, when knot numbers were 40–50% times higher than nowadays (Hagemeijer *et al.* 2004), *Dosinia* and other non-toxic alternatives were never very abundant (Wolff *et al.* 1993). Being a migratory species, it may thus very well be that the carrying capacity of the population is set elsewhere outside Banc d'Arguin (van Gils *et al.* 2009); for example in the Wadden Sea southward staging area, where commercial fisheries led to impaired (re)fuelling opportunities (Kraan *et al.* 2010).

It is yet unclear what determines the probability of high densities of *Dosinia*, but the negative correlation between annual averages of *Dosinia* and *Loripes* densities is remarkable (Fig. 8.4C; $r = -0.76$, $F_{1,6} = 8.30$, $P = 0.03$). As has been suggested elsewhere (van Gils *et al.* 2012), this indicates some form of competition between the two species. Alternatively, there may be differences in environmental conditions among years that steer the negative correlation. For example, observed dynamics in seagrass abundance may underlie this correlation (Folmer *et al.* 2012; Leyrer *et al.* 2012), with *Loripes* more strongly linked to seagrass habitat than *Dosinia* (Honkoop *et al.* 2008).

It is exciting to hypothesize about how defence strategies in one prey may have been selected for given the defence strategy in another prey. For example, is the bulkiness of *Dosinia* an evolutionary response to the toxicity of *Loripes*? The comparison between the DRM and the TDRM allows us to hypothesize along these lines: it suggests that toxicity of *Loripes* might have increased predation pressure on *Dosinia*, inducing, on an evolutionary time scale, extra armature in *Dosinia*. The reason behind this is that intake rates on *Dosinia* are much higher in the TDRM than in the DRM, especially in years of high *Loripes* abundance (see Appendix IV, Table IV.4). Under the DRM, which treats *Loripes* as if it was non-toxic, knots can reach their digestive constraint on *Loripes* only, leaving no room to add bulky *Dosinia*. By contrast, under the TDRM, many *Dosinia* can be added to the diet because intake rates on *Loripes* are reduced because of the toxicity constraint.

At the same time the evolution of thick-shelled armature in *Dosinia* may have led to increased predation pressure on *Loripes*, which in turn may have increased *Loripes*' toxicity. Namely, if *Dosinia* would have been relatively thinner shelled than *Loripes* (i.e., when $e_D/k_D > e_L/k_L$), then knots would prefer *Dosinia* over *Loripes* and would fully neglect *Loripes* in *Dosinia*-rich years. Note that the mechanism of enhanced predation pressure on one prey type as a consequence of induced anti-predator defence in the other prey type proposed here is a classic example of 'trait-mediated indirect interactions', which have received renewed attention in the ecological literature (Bolker *et al.* 2003; Werner & Peacor 2003; Yamauchi & Yamamura 2005).

With the chemoautotrophically fuelled *Loripes* being the top most abundant bivalve in the system, Banc d'Arguin can be classified as a chemosynthesis-based ecosystem (Dubilier, Bergin & Lott 2008). In contrast to Banc d'Arguin, most chemosynthesis-based ecosystems, such as deep-sea vents and seep systems, are renown for the their lack of predators (Carney 1994; Bergquist *et al.* 2003; Kicklighter, Fisher & Hay 2004). Possibly, such systems lack predators because of the overwhelming densities of toxic prey while non-toxic alternatives are not at hand (Tunnicliffe 1991). The presence of a suitable non-toxic prey may explain why predators are able to thrive at Banc d'Arguin. Hydrothermal vents and deep-sea cold seeps are geographically more isolated than seagrass beds, and also more hostile because of the limited availability of dissolved oxygen in the deep sea. Their isolated positions makes it costly for predators to switch between 'phototrophic' and 'chemotrophic' prey, which could be the reason that such systems are frequented little by predators originating from photosynthetic communities (Carney 1994; MacAvoy *et al.* 2008; Cordes, Becker & Fisher 2010). By contrast, in seagrass beds the difference between the anaerobic sulphidic and the aerobic non-toxic environment is just a matter of metres in a horizontal direction (bare versus seagrass mosaics (Honkoop *et al.* 2008; van der Heide *et al.* 2010)), or even centimetres when considered vertically (sulphide concentrations strongly increase in the first 12 cm of the sediment layer (van Gils *et al.* 2012)). This allows predators to 'make the best of both worlds' by adding toxic prey to their non-toxic diet as long as toxin levels do not exceed a given threshold. This mimics the problems recognized long ago for terrestrial herbivores, in which diet selection (Schmidt 2000; Dearing, Foley & McLean 2005), habitat use (Moore & Foley 2005), and fitness and population processes (DeGabriel *et al.* 2009) are governed by the occurrence of toxins in the form of secondary plant metabolites or as products from endosymbiotic relationships (Saikkonen *et al.* 1998; Verstraete *et al.* 2011). Our work seems to be the first to make similar problems apparent in a system with predators and prey rather than herbivores and plants.

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Appendix IV

Table IV.1 Year-specific input parameters used in the diet models: available numerical density (D; m^{-2}), AFDM$_{\mathrm{flesh}}$ (e; mg) and DM$_{\mathrm{shell}}$ (k; mg) of available *Loripes* (subscript L) and *Dosinia* (subscript D). Since the toxin constraint is defined in terms of maximally tolerable flesh mass intake rate, we expressed toxin contents $s_L = e_L$ and $s_D = 0$. Last row gives overall averages of e_L, e_D, k_L and k_D, which have been used to generate theoretical predictions in Figs. 8.4C and 8.5.

Year	D_L	D_D	e_L (= s_L)	e_D	k_L	k_D
2003	68.0	46.5	3.7	6.3	28.1	119.4
2004	23.9	1142.7	4.4	3.7	32.9	72.5
2005	93.5	223.3	9.8	1.1	74.8	22.9
2006	141.5	235.8	9.6	3.4	73.3	67.4
2007	376.3	98.7	5.2	2.6	39.4	52.9
2008	563.0	59.3	6.7	2.7	50.7	53.1
2009	725.5	8.3	4.6	3.4	35.2	63.4
2010	357.2	375.4	4.5	0.8	34.1	17.0
Average			6.1	3.0	46.1	58.6

Table IV.2 List of all candidate models tested for estimating apparent annual survival Φ and resighting probability p. Factors included in models were age (adult/juvenile), time (year as categorical variable), Time (year as continuous variable), tsm (time-since-marking), site (Abelgh Eiznaya/Baie d'Aouatif), TDRM (energy intake rate predicted by the TDRM) and DRM (energy intake rate predicted by the DRM). Model statistics listed are quasi-likelihood adjusted Akaike's Information Criterion corrected for small sample size (QAIC$_c$; noted at bottom of table for best model), number of parameters (np), QDeviance, ΔQAIC$_c$ and QAIC$_c$ weight.

No.	model	np	QDeviance	ΔQAIC$_c$	QAIC$_c$ weight
1	$\Phi_{\mathrm{age+TDRM}}\ p_{\mathrm{site+time}}$	12	941.44	0.00	0.23
2	$\Phi_{\mathrm{age+time}}\ p_{\mathrm{site+time}}$	18	929.33	0.01	0.23
3	$\Phi_{\mathrm{age*tsm+time}}\ p_{\mathrm{site+time}}$	19	927.65	0.35	0.19
4	$\Phi\mathrm{a}_{\mathrm{ge*tsm+TDRM}}\ p_{\mathrm{site+time}}$	13	941.05	1.63	0.10
5	$\Phi_{\mathrm{age+Time}}\ p_{\mathrm{site+time}}$	12	943.72	2.28	0.07
6	$\Phi_{\mathrm{age*tsm+Time}}\ p_{\mathrm{site+time}}$	13	942.20	2.77	0.06
7	$\Phi_{\mathrm{time}}\ p_{\mathrm{site+time}}$	17	934.13	2.78	0.06
8	$\Phi_{\mathrm{TDRM}}\ p_{\mathrm{site+time}}$	11	946.50	3.05	0.05
9	$\Phi_{\mathrm{Time}}\ p_{\mathrm{site+time}}$	11	949.67	6.21	0.01
10	$\Phi_{\mathrm{age+DRM}}\ p_{\mathrm{site+time}}$	12	949.69	8.25	0.00
11	$\Phi_{\mathrm{age*tsm+DRM}}\ p_{\mathrm{site+time}}$	13	948.87	9.45	0.00
12	$\Phi_{\mathrm{DRM}}\ p_{\mathrm{site+time}}$	11	957.21	13.76	0.00
13	$\Phi_{\mathrm{age}}\ p_{\mathrm{site+time}}$	11	967.03	23.57	0.00
14	$\Phi_{\mathrm{age*tsm}}\ p_{\mathrm{site+time}}$	12	967.00	25.56	0.00
15	$\Phi_{\mathrm{constant}}\ p_{\mathrm{site+time}}$	10	973.76	28.30	0.00
QAIC$_c$ = 7400.05					

Table IV.3 Parameter estimates obtained by model averaging. Presented are estimate, standard error (SE), and upper and lower 95% confidence interval (CI) for apparent survival Φ (Φ^1 = adult survival in year after capture, Φ^{2+} = adult survival in subsequent years, Φ^{juv} = juvenile survival in the year after capture) and recapture probability p (subscript A for site Abelgh Eiznaya and B for site Baie d'Aouatif).

	year	estimate	SE	lower CI	upper CI
apparent survival					
Φ^{2+}	2004	0.92	0.05	0.77	0.98
Φ^{2+}	2005	0.83	0.03	0.76	0.88
Φ^{2+}	2006	0.88	0.03	0.80	0.93
Φ^{2+}	2007	0.83	0.02	0.78	0.87
Φ^{2+}	2008	0.78	0.03	0.72	0.83
Φ^{2+}	2009	0.76	0.03	0.69	0.81
Φ^1	2003	0.82	0.05	0.72	0.90
Φ^1	2004	0.92	0.05	0.76	0.98
Φ^1	2005	0.82	0.03	0.75	0.87
Φ^1	2006	0.87	0.04	0.78	0.93
Φ^1	2007	0.82	0.03	0.76	0.87
Φ^1	2008	0.77	0.03	0.69	0.83
Φ^1	2009	0.75	0.04	0.67	0.81
Φ^{juv}	2003	0.77	0.06	0.64	0.87
Φ^{juv}	2004	0.89	0.07	0.68	0.97
Φ^{juv}	2005	0.76	0.05	0.67	0.84
Φ^{juv}	2006	0.83	0.05	0.71	0.91
Φ^{juv}	2007	0.77	0.05	0.66	0.85
Φ^{juv}	2008	0.70	0.04	0.61	0.78
Φ^{juv}	2009	0.68	0.05	0.57	0.78
resighting probability					
p_A	2003	0.30	0.04	0.23	0.38
p_A	2004	0.34	0.03	0.29	0.40
p_A	2005	0.47	0.03	0.41	0.52
p_A	2006	0.50	0.03	0.45	0.56
p_A	2007	0.56	0.03	0.50	0.60
p_A	2008	0.52	0.03	0.47	0.57
p_A	2009	0.65	0.03	0.60	0.71
p_B	2003	0.24	0.04	0.18	0.32
p_B	2004	0.28	0.03	0.21	0.35
p_B	2005	0.40	0.04	0.32	0.47
p_B	2006	0.43	0.03	0.37	0.50
p_B	2007	0.48	0.03	0.42	0.55
p_B	2008	0.44	0.03	0.39	0.50
p_B	2009	0.58	0.03	0.52	0.65

Table IV.4 Output from the two diet models for each of the study years, with p referring to the probability that a prey item is accepted upon encounter for *Loripes* (subscript L) or *Dosinia* (subscript D), Y giving the predicted energy intake rate (mg AFDM$_{\mathrm{flesh}}$ s^{-1}), Y_L and Y_D giving the absolute contributions of respectively *Loripes* and *Dosinia* to Y, $X = c$ indicating whether the digestive constraint is met (y) or not (n), and $Z = q$ indicating whether the toxin constraint is met or not.

	DRM						TDRM						
Year	p_L	p_D	Y	Y_L	Y_D	$X = c$	p_L	p_D	Y	Y_L	Y_D	$X = c$	$Z = q$
2003	1.00	1.00	0.21	0.10	0.11	n	1.00	1.00	0.21	0.10	0.11	n	n
2004	1.00	0.16	0.29	0.04	0.25	y	1.00	0.16	0.29	0.04	0.25	y	n
2005	1.00	1.00	0.41	0.33	0.09	n	0.30	1.00	0.19	0.10	0.09	n	y
2006	1.00	0.21	0.57	0.51	0.06	y	0.20	0.74	0.32	0.10	0.22	y	y
2007	0.99	0.00	0.67	0.67	0.00	y	0.14	1.00	0.20	0.10	0.10	n	y
2008	0.50	0.00	0.67	0.67	0.00	y	0.07	1.00	0.16	0.10	0.06	n	y
2009	0.58	0.00	0.67	0.67	0.00	y	0.08	1.00	0.11	0.10	0.01	n	y
2010	1.00	0.53	0.58	0.53	0.05	y	0.18	1.00	0.20	0.10	0.10	n	y

Table IV.5 As Table IV.2, but having our 2004 benthos data replaced by those from Honkoop *et al.* (2008).

model	np	QDeviance	ΔQAIC$_c$	QAIC$_c$ weight
$\Phi_{\mathrm{age+TDRM}}\ p_{\mathrm{site+time}}$	12	939.39	0.00	0.38
$\Phi_{\mathrm{age*tsm+TDRM}}\ p_{\mathrm{site+time}}$	13	938.89	1.52	0.18
$\Phi_{\mathrm{age+time}}\ p_{\mathrm{site+time}}$	18	929.33	2.06	0.14
$\Phi_{\mathrm{age*tsm+time}}\ p_{\mathrm{site+time}}$	19	927.65	2.40	0.12
$\Phi_{\mathrm{TDRM}}\ p_{\mathrm{site+time}}$	11	944.81	3.41	0.07
$\Phi_{\mathrm{age+Time}}\ p_{\mathrm{site+time}}$	12	943.72	4.33	0.04
$\Phi_{\mathrm{age*tsm+Time}}\ p_{\mathrm{site+time}}$	13	942.20	4.83	0.03
$\Phi_{\mathrm{time}}\ p_{\mathrm{site+time}}$	17	934.13	4.83	0.03
$\Phi_{\mathrm{Time}}\ p_{\mathrm{site+time}}$	11	949.67	8.26	0.01
$\Phi_{\mathrm{age+DRM}}\ p_{\mathrm{site+time}}$	12	952.20	12.81	0.00
$\Phi_{\mathrm{age*tsm+DRM}}\ p_{\mathrm{site+time}}$	13	951.64	14.27	0.00
$\Phi_{\mathrm{DRM}}\ p_{\mathrm{site+time}}$	11	958.99	17.59	0.00
$\Phi_{\mathrm{age}}\ p_{\mathrm{site+time}}$	11	967.03	25.62	0.00
$\Phi_{\mathrm{age*tsm}}\ p_{\mathrm{site+time}}$	12	967.00	27.61	0.00
$\Phi_{\mathrm{constant}}\ p_{\mathrm{site+time}}$	10	973.76	30.35	0.00
QAIC$_c$ = 7398.00				

Section IV.1

Sensitivity analysis with respect to benthos sampling. Here we explore whether our conclusion that the TDRM is the best-supported model has been affected by our spatially inconsistent sampling program. We do so by replacing our benthos data collected in 2004 by those from an independent study by Honkoop *et al.* (2008), also carried out in 2004 in our study area. Honkoop *et al*. (2008), who sampled stratified with respect to habitat, found lower densities in bare (219.4 and 60.0 m^{-2} for *Dosinia* and *Loripes*, respectively) than in seagrass habitat (316.1 and 338.9 m^{-2} for *Dosinia* and *Loripes*, respectively). Correcting these densities for species-specific availability fractions (0.73 in *Dosinia* and 0.70 in *Loripes* (van Gils *et al.* 2012)) and for the fact that on average at Banc d'Arguin bare-seagrass habitat occurs in a 20–80% ratio (Wolff & Smit 1990), leads to the following available densities of *Dosinia* and *Loripes*: 216.6 and 198.2 m^{-2}. Assuming the same values for e_1, e_2, k_1 and k_2 as we found in our study in 2004 (Table IV.1) predicts that knots would face both their toxin and their digestive constraint in 2004 at an energy intake rate Y of 0.45 (DRM) and 0.33 mg AFDM$_{\text{flesh}}$ s^{-1} (TDRM; compared to 0.29 mg AFDM$_{\text{flesh}}$ s^{-1} for both DRM and TDRM when using our own 2004 benthos data; Table IV.4).

The outcome of the survival analyses is hardly affected by this data replacement (Table IV.5). There is an even stronger support for the models including TDRM (cumulative QAIC$_c$ weight: 0.63), and again no support for the DRM models (cumulative QAIC$_c$ weight: 0.00). Also the models including time as a categorical factor (cumulative QAIC$_c$ weight: 0.29) and time as a continuous variable (cumulative QAIC$_c$ weight: 0.08) found little support.

Also the outcomes of the diet statistics were unaffected. DRM still overestimated the proportion of *Loripes* in the diet (t = –4.70, df = 4, P = 0.01), while TDRM diets still matched the observed diets (t = –1.61, df = 4, P = 0.18).

Section IV.2

This section explains the theoretical basics underneath Fig. 8.4C, with *Loripes* being denoted by prey type 1 and *Dosinia* by prey type 2.

Minimal *Loripes* density (D_q) at and above which toxin constraint q is achieved:
Toxin intake rate Z is given by:

$$Z = \frac{a_1 D_1 s_1 + a_2 D_2 s_2}{1 + a_1 D_1 h_1 + a_2 D_2 h_2} \qquad \text{(IV.1}a\text{)}$$

Since type 2 is not poisonous in our case (i.e. $s_2 = 0$) equation IV.1*a* simplifies to:

$$Z = \frac{a_1 D_1 s_1}{1 + a_1 D_1 h_1 + a_2 D_2 h_2} \qquad \text{(IV.1}b\text{)}$$

Toxin constraint q is met when:

$$Z = q \qquad \text{(IV.2)}$$

Solving equations IV.1*b* and IV.2 for D_1 yield type 1's density D_q at and above which q is reached:

$$D_q(\text{type 1}) = \frac{D_2 a_2 h_2 q + q}{a_1(s_1 - h_1 q)} \qquad \text{(IV.3)}$$

Note that D_q increases slightly with an increase in D_2 (solid line in Appendix-Fig. IV.1), which is due to increasing time loss to handling type 2, which goes at the expense of available search time and thus hampers the intake rate on type 1 at a given density D_1.

Minimal density (D_c) of *Loripes* or *Dosinia* at and above which digestive constraint c is achieved:
Ballast intake rate X is given by:

$$X = \frac{a_1 D_1 k_1 + a_2 D_2 k_2}{1 + a_1 D_1 h_1 + a_2 D_2 h_2} \qquad \text{(IV.4)}$$

Digestive constraint c is met when:

$$X = c \qquad \text{(IV.5)}$$

Solving equations IV.4 and IV.5 for N_1 yields type 1's density N_c at and above which c is reached:

$$D_c(\text{type 1}) = \frac{D_2 a_2 h_2 c - D_2 a_2 k_2 + c}{a_1(k_1 - h_1 c)} \qquad \text{(IV.6)}$$

Vice versa, D_c can also be expressed for type 2 by solving equations IV.4 and IV.5 for D_2:

$$D_c(\text{type 2}) = \frac{D_1 a_1 h_1 c - D_1 a_1 k_1 + c}{a_2(k_2 - h_2 c)} \qquad \text{(IV.7)}$$

Note that D_c decreases with an increase in D1 (dashed line in Appendix-Fig. IV.1), which is because the more of type 1 is ingested, the less of type 2 needs to be ingested in order to reach the digestive constraint.

Minimal densities of *Loripes* (D_1^*) and *Dosinia* (D_2^*) at which both constraints q and c are met:

These densities are found by equating equation IV.3 with equation IV.6 and solving for D_1 and D_2, yielding:

$$D_1^* = \frac{k_2 q}{(a_1 k_2 - a_1 c h_2) s_1 + (a_1 h_2 k_1 - a_1 h_1 k_2) q} \qquad \text{(IV.8)}$$

$$D_2^* = \frac{c s_1 - k_1 q}{(a_2 k_2 - a_2 c h_2) s_1 + (a_2 h_2 k_1 - a_2 h_1 k_2) q} \qquad \text{(IV.9)}$$

Density 'regions' with or without constraint(s):

As visualized in Appendix-Fig. IV.1, both constraints operate when $D_1 > D_1^*$ and $D_2 > D_2^*$. Only the toxin constraint operates when $D_1 > D_q$ and $D_2 < D_2^*$. Only the digestive constraint acts when $D_1 < D_1^*$ and $D_2 > D_c$. None of the constraints act when $D_1 < D_q$ and $D_2 < D_c$. Note that within these larger regions there are two interesting 'subregions'. First, there is a subregion where D_1 is high enough for the digestive constraint to be met – however the existence of the toxin constraint precludes this. This is where $D_1 > D_c$ and $D_2 < D_2^*$. Second, there is subregion where the existence of the digestive constraint makes the toxin constraint being faced at a lower density than D_q, which happens because the slowing down effect of handling type 2 prey has disappeared due to digestive constraint c setting a limit to the handling frequency. This is where $D_1 < D_q$ and $D_1 > D_1^*$ (where by definition $D_2 > D_2^*$).

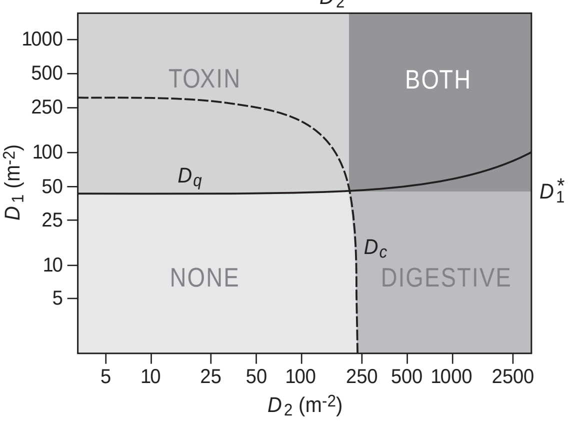
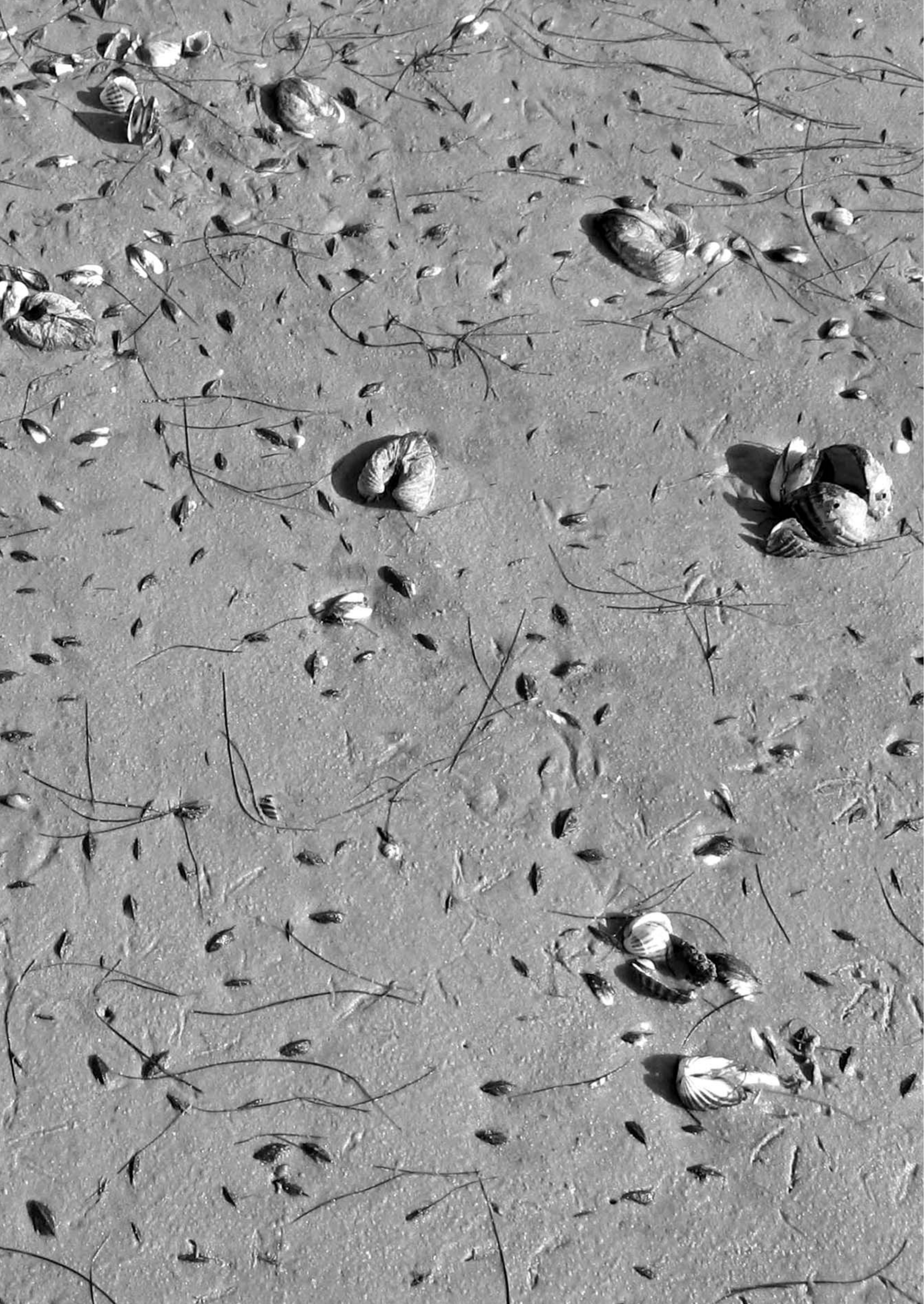


Figure IV.1 The basis underlying Fig. 8.4C showing how we derived critical density thresholds above which the toxin and the digestive constraints operate (for the average year, i.e. by taking as inputs the means of e_L, e_D, k_L, and k_D listed in the last row of Table IV.1). Note that axes are log-transformed, making D_q and D_c curved instead of straight lines.



Chapter 9

Density-dependent growth in three tropical intertidal bivalves: importance of feeding style, habitat and season

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Abstract

1. Density-dependent processes are key in population regulation. This study extends previous assessments of Levinton's (1972) prediction that in marine biota benthic deposit feeders should be limited by food availability and benthic suspension feeders should not. Furthermore, we will provide the first evidence for density-dependent growth in chemosymbiotic bivalves.

2. We carried out density manipulations of benthic communities in contrasting intertidal habitats (bare sandy sediments vs. seagrass-covered mud) and measured bivalve growth rates in two contrasting seasons, namely autumn–winter (the season of slow growth) and spring–summer (the season of fast growth).

3. The study was carried out in the tropics (Banc d'Arguin, Mauritania, 20°N) on the locally abundant bivalve species *Senilia senilis* (Arcidae), *Dosinia isocardia* (Veneridae) and *Loripes lucinalis* (Lucinidae), of which the first two are considered suspension feeders and the third is a facultative mixotroph, which is able to suspension-feed, but which mainly lives on sugars produced by its endosymbiotic sulphide-oxidizing gill-bacteria.

4. *In situ* mollusc densities were manipulated and the growth rates of individually tagged *Senilia*, *Dosinia* and *Loripes* clams from each treatment (density doubled vs. control) were measured across the seasons.

5. Von Bertalanffy's growth constant k (day^{-1}) of suspension-feeding *Dosinia* and chemosymbiotic *Loripes* was density dependent in both seasons, but no such effect could be demonstrated for suspension-feeding *Senilia*. All three species showed significantly reduced growth during the autumn–winter season.

6. The inference that density- and season-dependent processes regulate growth in chemosymbiotic bivalves is novel, but we believe it may be a general phenomenon in coastal chemosymbiotic communities. Habitat quality may explain why density-dependent growth is observed in suspension-feeding *Dosinia* inhabiting seagrass sediments, where suspended food availability seems to be limited by seagrass reducing current velocities, and not in suspension-feeding *Senilia* inhabiting bare sediments.

7. Potential cascading trophic effects on density-dependent growth rates in *Loripes* and *Dosinia* by their main predator, the molluscivore red knot (*Calidris c. canutus*), a population in decline, are discussed.

Introduction

Competition for resources provides a key density-dependence process, that contributes to the relative constancy of population sizes (Begon, Townshend & Harper 2006). For organisms serving as food for other organisms (and most do), density-dependent processes embody another layer of implication. By thinning prey that are subject to intraspecific competition, predators could boost food supplies by their own predatory acts (de Roos *et al.* 2007; van Leeuwen, de Roos & Persson 2008). Density-dependent processes thus have cascading trophic effects and contribute to the structuring of interaction webs (Persson *et al.* 2007; van Leeuwen, de Roos & Persson 2008; Olff *et al.* 2009). However, it is interesting to determine under which biological circumstances density-dependent processes have a greater or smaller influence on population size and overall community structure (Sih *et al.* 1985; Gurevitch, Morrison & Hedges 2000; Chase *et al.* 2002).

The presence or absence of density dependence is most easily studied in accessible organisms that are sedentary, and of which densities can be manipulated. Bivalves living in soft-sediment intertidal foreshores have the advantage that they are easy to get to, can be individually marked, and have biological properties such as growth, condition, reproductive investment and survival relatively easily and accurately quantified (Wilson 1991). An effective integration of natural history insights led Levinton (1972) to formulate predictions on degrees of inter- and intraspecific competition for food, and on population dynamics, of the contrasting guilds of suspension- and deposit-feeding marine organisms. Levinton proposed that deposit feeders, relying on rather constant and partially self-renewing food supplies on and in the upper layer of sediments, should be food-specialists occurring in densities at which they are limited by food availability. In contrast, suspension feeders, relying on the notoriously seasonally and locally variable phytoplankton in the overlying water, should be food-generalists occurring in variable densities not closely regulated by food availability. The clarity of his predictions spawned a rich portfolio of descriptive and manipulative tests in benthic marine invertebrates, mostly conducted at temperate coastal systems (e.g., Peterson 1982; Olafsson 1986; Peterson & Black 1987; Peterson & Beal 1989; Kamermans *et al.* 1992; Peterson & Black 1993; Beal, Parker & Vencile 2001; Wenngren & Olafsson 2002; Beal 2006; Fodrie *et al.* 2007).

As envisaged by Levinton, when put to the test, deposit feeders showed decreased growth rates at increased densities (e.g., Branch & Branch 1980; Kamermans *et al.* 1992; Wenngren & Olafsson 2002). Most studies on suspension-feeding bivalves showed no density-dependent growth (e.g., Kamermans *et al.* 1992; Beal, Parker & Vencile 2001). In a particularly elegant study, Ólafsson (1986) confirmed this pattern within a single species: growth of the tellinid bivalve *Macoma balthica* was density dependent in muddy sands where deposit feeding predominated, but was density independent in sandy sediments where suspension feeding was the rule. However, some studies did report density-dependent reductions in growth rates of suspension feeders at very high densities (e.g., Peterson 1982; Peterson & Black 1987; Peterson & Beal 1989; Vincent, Joly & Brassard 1989; Jensen 1993; Peterson & Black 1993). This indicates that the presence or absence of density-dependent growth not only reflects feeding style, but that ecological context also plays an important role. Context includes a precision of characteristics and structure of the overlying water mass and benthic habitat or geographical setting, and could also include time of the year: one would expect density-dependent reductions in bivalve growth rates to be better detectable in the season of fastest growth.

This study may be seen as 'just' another test of Levinton's (1972) predictions, but we aim to take it a few steps further. First, we carried out experimental manipulations of natural densities of soft-sediment suspension-feeding communities in a novel setting in the tropics (20°N) and in contrasting intertidal habitats (bare coarse sediment vs. seagrass-covered mud). Secondly, we measured individual growth rates of three locally abundant bivalve species in two contrasting seasons, between October 2007 and April 2008 (the season of slow growth; hereafter called winter) and between April 2008 and November 2008 (the season of fast growth; hereafter called summer). Thirdly, our study includes the first assessment of density dependence of growth rate in a species that mainly lives of the carbon products provided by chemoautotrophic endosymbiotic bacteria.

In our tropical study area, Banc d'Arguin, Mauritania, deposit feeders are rare, but *Senilia senilis* (Arcidae; *Senilia* hereafter), *Dosinia isocardia* (Veneridae; *Dosinia* hereafter) and *Loripes lucinalis* (Lucinidae; *Loripes* hereafter) are common, contributing to ~80% of the overall numbers of benthic animals (Honkoop *et al.* 2008), with *Senilia* occurring mostly in bare sands (242 ind. m^{-2} in bare vs. 59 ind. m^{-2} in seagrass; Honkoop *et al.* 2008), and *Dosinia* and *Loripes* occurring mostly in seagrass-covered sediments (respectively 219 and 60 ind. m^{-2} in bare and 316 and 339 ind. m^{-2} in seagrass; Honkoop *et al.* 2008). *Senilia* and *Dosinia* can be considered suspension feeders (Honkoop *et al.* 2008), whereas *Loripes* is a facultative mixotroph, which is capable of suspension feeding, but to a large extent obtains its nutrition through a symbiosis with sulphide-oxidizing bacteria living inside its gills (Herry, Diouris & Le Pennec 1989; Johnson, Diouris & Le Pennec 1994, Chapter 2). In this symbiotic association, the lucinid host favours bacterial chemosynthesis by facilitating the supply of sulphide, CO_2 and oxygen. In exchange, the bacterial symbionts fix carbon, fuelling their own energetic and biosynthetic needs, in addition to those of their host (Stewart, Newton & Cavanaugh 2005).

First, we predict density-dependent depression in growth rate in 'chemosymbiotic' *Loripes*, because the bacterial chemosynthesis of their food is limited by the transport of sulphide, oxygen and CO_2 from the local environment to the gill-inhabiting sulphide-oxidizing symbionts by the bivalve host. We predict no such effects on growth rates of suspension feeders (*Senilia* and *Dosinia*) that obtain their food (e.g., resuspended benthic diatoms) from the overlying water. Second, we predict interactions between time of the year and the experimental effect of manipulating local *Loripes* densities – this effect should be more detectable during the summer season when *Loripes* has faster growth rates (see Chapter 7) compared to the winter season. Third, we predict interactions between habitat (muddy seagrass sediments vs. bare sandy sediments) and the manipulated local *Loripes* density, with the density-dependent effect being smaller in the more muddy and organic-rich sediments of seagrass beds (Honkoop *et al.* 2008; Folmer *et al.* 2012), where growth conditions for the endosymbiont–lucinid bivalve association should be more optimal, because of both higher sulphide pore-water concentrations and radial oxygen release from the seagrass roots (van der Heide *et al.* 2012).

Tests of these predictions in a tropical intertidal system for both suspension-feeding and chemosymbiotic bivalve communities not only expand considerably the scale of tests on the influence of local density on growth of bivalves from different feeding guilds, but also contribute to an improved mechanistic understanding of the processes by which interactions involving time and habitat effects modify bivalve growth.

Methods

Study site

The study area is the Iwik region (Fig. 9.1), which is an accessible part of the intertidal area of Banc d'Arguin (19º60'–19º33'N, 16º33'–16º35'W) off the coast of Mauritania. This intertidal ecosystem is characterized by tidal flats of which ~80% are covered by seagrass (mainly *Zostera noltii* Hornemann; Wolff and Smit, 1990). Banc d'Arguin is an important wintering site for migratory shorebirds using the East Atlantic Flyway, hosting more than two million individuals in winter (Altenburg *et al.* 1982). Previous studies have indicated that this intertidal area is mainly a benthic-diatom-dominated trophic system with low contributions of phytoplankton, macrophytes and epiphytes to the food web (Sevrin-Reyssac 1984; Wolff *et al.* 1993; Honkoop *et al.* 2008; see also Chapter 2). Being adjacent to the Sahara desert, Banc d'Arguin does not receive freshwater inflow from rivers and precipitation is limited to occasional thunderstorms that occur at irregular intervals, sometimes several years apart (Wolff & Smit 1990).

In the Iwik region, seawater temperature varies between ~20°C in winter (i.e., January) and ~30°C in late summer (i.e., September) and salinity ranges from 40–43‰ in February-April and from 42–43‰ in September (Wolff & Smit 1990; Lavaud *et al.* 2013). The tide is semi-diurnal and the tidal range is 1.5–2 m.

Experimental design

To avoid artefacts that may be imposed by commonly used enclosures experiments (see Appendix V, Section V.1), we used an alternative experimental set-up to manipulate clam densities while keeping growing conditions as natural as possible, as will be described below.

The study area was divided into seven sub-regions (Fig. 9.1) which in turn were subdivided into annuli with an outer radius of 200 m and an inner radius of 100 m. Within each annulus (hereafter named station), 10 sampling sites were randomly selected. Our sampling procedure thus yielded 70 sampling sites.

Between 13 October and 5 November 2007, benthic samples were taken at each sampling site during low tide ($N = 70$ benthic samples). A benthic sample consisted of a 15-cm-high PVC ring (diameter, 30 cm) that was pushed 10 cm into the sediment from which sediment was collected to a depth of 10 cm, using a small shovel. The content was sieved over

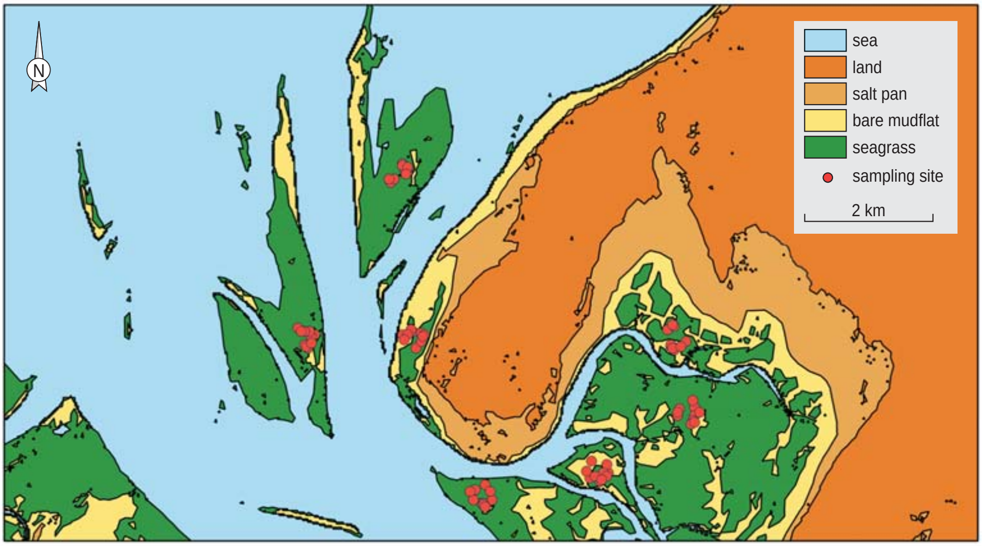


Figure 9.1 Position of the sampling sites on the mudflats surrounding Iwik Peninsula, Banc d'Arguin, Mauritania.

a 2.8-mm mesh and the retained material was transported to the field station near Iwik. Here, all living molluscs (i.e., mainly bivalves and to a lesser extent gastropods) were separated from the matrix of seagrass remains and shells fragments. Individuals of *Senilia*, *Dosinia* and *Loripes*, respectively were haphazardly selected and shell height (H_1) was measured to the nearest 0.1 mm using a digital calliper. Subsequently, all measured clams were individually tagged with a uniquely labelled Hallprint glue-on shellfish tag (type 'FPN 4 mm circle tag' or type 'FPN 8x4 mm oval tag'). Before redeployment, all specimens per sample were stored at ~25°C, while being covered with a layer of moist *Zostera noltii* leaves to protect them from desiccation. Within 24 h of collection all tagged and untagged molluscs from the same sample were replaced in their natural environment. As sampling inevitable leads to disruption of the benthic system, specimens were relocated at an undisturbed site at 5 m distance of the site where they were initially sampled. The site of replacement was marked with two PVC sticks that were placed 50 cm apart. Exactly in between the two poles a 30 cm diameter PVC ring was placed on top of the sediment, and all tagged and untagged molluscs collected near this site were replaced by gently pushing them in to the sediment within the enclosed area to a depth of 1 cm, after which the PVC ring was removed. Assuming that the composition of the benthic community does not differ at a 5 m distance, the replacement of all molluscs from one sample in a surface area that is equal to the surface area from which they were sampled will result in densities that are roughly twice the natural densities of molluscs at this site.

For those of the three bivalve species (i.e., *Senilia*, *Dosinia* and *Loripes*) of which individuals were tagged and densities were manipulated at a particular sampling site, we collected 2–6 additional specimens by digging into the sediment using a small shovel. Of these specimens, shell height was also measured (precision 0.1 mm) after which they were individually tagged as described above. In contrast to the treatment where ambient densities were doubled, these tagged specimens were each placed 1 m apart and within 5 m of the site where ambient densities were doubled. Assuming that ambient mollusc densities were not greatly changed by the addition of a single individual per m^2, these sites were considered as our control treatments where natural bivalve densities were not manipulated. These control sites were again marked by two short PVC sticks placed 50 cm apart (the middle point between two short PVC sticks marked the spot where the tagged specimen was relocated).

Between 13 April 2008 and 4 May 2008 we sampled our experimental relocation sites, by pushing a 30 cm diameter PVC ring 15 cm deep into the sediment at each site (the PVC ring was placed in the centre of the two PVC sticks that marked each site) and scooping out the sediment to 15 cm depth using a small shovel. To reduce sampling effort, the control sites to which only one tagged clam was allocated, were sampled by using a smaller sediment core (diameter, 15 cm) taken to 15 cm depth. Each sample was sieved over a 2.8-mm mesh and the material retained was put in a plastic bag and transported to the scientific field station near Iwik. Here, all living clams were sorted per sample and when a tagged clam was recovered, its shell height (H_2) was measured again (precision 0.1 mm). At each sampling, tagged clams were either found alive, found dead as empty shells, or missing. Missing clams were a consequence of sampling error, emigration, removal by scavengers, post-mortem transport, or predation. In many occasions we recaptured single tags in our samples that were not attached to a clam anymore. Because we could not determine whether these tags belonged to clams that were alive, dead or missing from our study plot, we labelled their fate as ‘unknown’.

To investigate seasonal effects on density-dependent processes, we repeated this mark-recapture experiment between April and November 2008 at the same study sites. Subsequently, from 13 April 2008 to 4 May 2008, clams were collected, measured and individually tagged as described above, while from 20 October 2008 to 14 November 2008 the relocation-sites were sampled.

A total of 1,473 clams were tagged (*Senilia senilis*, $N = 541$; *Dosinia isocardia*, $N = 368$; *Loripes lucinalis*, $N = 564$). For each species, the total number of tagged clams and mean shell height (mm) per experimental period and per treatment is presented in Table V.1 (see Appendix V), as well as their fate (percentage of tagged clams recovered alive, recovered dead as empty shells, found missing or of which the fate was unknown). Given the large treatment, season and species-specific differences in percentage of clams labelled as ‘unknown’ (see Appendix V, Table V.1), and the unknown cause of these differences, we refrained from investigating the effect of season and treatment on recapture rate per bivalve species.

Site-specific habitat characteristics

Between 13 April and 2 May 2008 (i.e., half-way our study period), at each site a seagrass sample was taken using a circular PVC core (diameter,70 mm) that was pushed 10 cm into the sediment. The content was sieved over a 500-µm mesh. The material retained on the sieve was stored in a plastic bag, frozen at -18°C and transported to the Netherlands, where for each sample all living seagrass parts (i.e., leaves, roots and rhizomes) were sorted. The ash-free dry mass (AFDM) of all living seagrass parts was determined via the loss-on-ignition method. Samples were dried at 60°C for a minimum of 72 h, weighed and then incinerated at 550°C for 4 h after which the remaining ashes were weighed again. The difference between the first and the second measurements gives the AFDM of the living seagrass parts in the sample (AFDM seagrass, in g m^{-2}).

When taking a seagrass sample at each site, an additional sediment sample was taken to a depth of 10 cm by pressing a PVC tube (diameter, 1 cm) into the sediment. The sediment sample was also stored in a plastic bag, frozen at -18°C and transported to the Netherlands, where samples were freeze-dried and grain size distribution of each sample was determined using a particle-size analyser (Beckman Coulter Model LS 230). From the grain size distribution the median (median grain size, MGS) was calculated. For details on grain size measurements see Honkoop *et al.* (2008).

Bivalve growth rate

For each of the three species, the mean initial size of recaptured bivalves varied between our treatments (see Appendix V, Table V.1). To remove the effects of initial size on the magnitude of individual growth increments, we fitted Von Bertalanffy's growth function (VBGF) to our data, a commonly used equation when modeling indeterminate bivalve growth. In this function, growth rate dH/dt declines with an increase in shell height H_t in the following way:

$$\frac{dH}{dt} = k\,(H_\infty - H_t) \qquad (1)$$

where H_∞ is the mean maximum size and k is the growth constant. To estimate the growth constant k from tag–recapture data, the traditional VBGF has to be modified using the derivation of Fabens (1965) increment model:

$$H_2 = H_\infty - (H_\infty - H_1)e^{-k\Delta t} \qquad (2)$$

where k is the estimated growth constant and H_1 and H_2 are defined as the shell heights at time of marking (t_1) and recapture (t_2), respectively, Δt as the time interval in days (i.e., $t_2 - t_1$) and is the mean maximum shell height. Rewriting this equation gives:

$$k = -\frac{\ln\left(\dfrac{H_\infty - H_2}{H_\infty - H_1}\right)}{\Delta t} \qquad (3)$$

We used the obtained growth constant k (day^{-1}) for each recaptured specimen as an index of growth in our analyses, which can be viewed as the daily intrinsic rate of growth from an exponential growth equation.

Statistical analysis

As sediment grain size and seagrass biomass were highly correlated ($F_{1,68} = 21.0$, $P < 0.001$), we used a principle component analysis to derive a single variable describing the habitat characteristics at a specific sampling site (see Appendix V, Fig. V.1). The first principle component (PC1) explained 74% of the proportion of the variance in sediment grain size and seagrass biomass, and was used in the statistical models to describe the sampling site-specific habitat characteristics.

We used linear mixed effects models to investigate whether density treatment (control or initial density doubled), season (winter or summer) and habitat (PC1, continuous variable) and their two-way interactions explained variation in growth constant k (day^{-1}) of the three focal species. Tidal exposure is a factor of primary importance to the growth of suspension feeders, as it limits feeding time (Peterson & Black 1987). However, due to the lack of site-specific data on tidal elevation, we were unable to include the effect of tidal elevation in our models. Due to the nested structure of the data, station and site (nested in station) were included as random effects. We compared all possible combinations of these explanatory variables and used model selection based on the Akaike Information Criterion adjusted for small sample size (AIC$_c$; Burnham & Anderson 2002) to determine the most parsimonious model. ΔAIC$_c$ values were used to compare the relative explanatory value of the models, with ΔAIC$_c$ defined as the difference between the AIC$_c$ value of the best-fitting model and each respective model in the set. AIC$_c$ weights were also calculated to provide a relative weight of evidence for each model (Burnham & Anderson 2002). The effect of a parameter was considered significant when adding this parameter to the model reduced the AIC$_c$ with at least two points.

We tested for heterogeneity in the residuals following the procedure described by Zuur *et al.* (2009), by comparing models that described the variance as different functions of the explanatory variables. In addition, we assessed whether the variance was described as a function of initial size (H_1), as this would indicate that the assumed H_∞ was inappropriate. Because the Von Bertalanffy growth model assumes that growth rate decreases linearly with clam size until growth becomes 0 at H_∞, any large clams that approach H_∞ and grew relatively fast will have a disproportionately large estimated k. When this is the case, a model where the variance is described as a positive (e.g., power or exponential) function of H_1 will be supported over a model where the variance is not a function of H_1, which indicates that H_∞ was chosen too small. Similarly, when H_∞ is chosen too large, any small clams that grew relatively fast will have a disproportionately large estimated k. Therefore, by changing the value of until the most parsimonious variance function no longer includes H_1 provides a method to determine the appropriate value for H_∞. This turned out to be at 11.3, 17.1 and 77.0 mm for *Loripes*, *Dosinia* and *Senilia*, respectively. Using these values for H_∞, there was still evidence that variance was season-dependent in all three focal species for which we corrected using a 'varIdent' structure (Pinheiro & Bates 2000; Zuur *et al.* 2009), which allows different error variances for different factor levels. This variance structure was retained when investigating the statistical support for any of the fixed effects. As there is some individual variation around H_∞ we performed a sensitivity analysis with respect to the maximum value of H_∞. Note that this sensitivity analysis is only performed for H_∞ values larger than those determined for each species, as for smaller values we will have to incorporate a variance structure described as a function of initial size, which will result in erroneous predictions of *k*.

All analyses were performed in program R (R Development Core Team 2013, version 3.0.0). For linear mixed effects models, the R-package 'nlme' (Pinheiro *et al.* 2013) was used. Models including all one- and two-way interactions of main effects were compared simultaneously based on $\mathrm{AIC_c}$, using the R-package 'MuMIn' (Bartoń 2012).

Results

Live recaptured clams ($N = 330$) were distributed over 68 of the 70 experimental sites, with *Senilia* specimens being restricted mainly to bare sandy sediment sites with relatively low PC1 values, and clams of *Dosinia* and *Loripes* being restricted to more muddy seagrass-covered sites with relatively high PC1 values (Fig. 9.2).

For *Senilia*, the most parsimonious model to account for variations in growth rate included an effect of season only (Table 9.1). Compared to summer, growth constant *k* in winter was reduced by 45.8% (95% CI of 32.2–59.4%; Table 9.2, Fig. 9.3A). Although the model including both season and density treatment as fixed effects was best supported (accumulated model weight: 0.27; Table 9.1), it added an extra parameter (complexity) to the model without $\mathrm{AIC_c}$ being reduced by at least 2 points (Table 9.1), and should thus be less favoured than the model that only included season as a parameter. The effect of doubling local clam density would be to reduce *k* (averaged over summer and winter) for *Senilia* by 17.2% (95% CI of -2.8–37.2%; Fig. 9.3A).

The most parsimonious models to account for growth of *Dosinia* and *Loripes*, respectively, included effects of season and density treatment (Table 9.1). For *Dosinia*, *k* in winter was reduced by 75.5% (95% CI of 66.7–84.3%; Table 9.2, Fig. 9.3B) compared to summer calculated for control density treatments, while doubling the local clam density reduced *k*

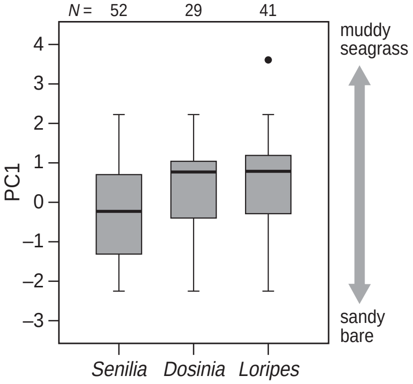


Figure 9.2 First principle component (PC1) values for sampling sites where at least one tagged specimen was recaptured given per species (i.e., *Senilia senilis*, *Dosinia isocardia* and *Loripes lucinalis*). Box-and-whisker plots give the median (horizontal line inside the box), interquartile range (box), range (bars), and outliers (black dot). Numerals above Box-and-whisker plots indicate sample sizes.

Table 9.1 Model selection results for growth constant *k* (day^{-1}) as a function of density treatment (t), season (s) and habitat (h; modeled as a continuous PC1 variable) and all possible two-way interactions for *Senilia senilis*, *Dosinia isocardia* and *Loripes lucinalis*. The most parsimonious model is shown in bold. K denotes the number of parameters. Only models with a model weight of >0.01 are shown.

	K	logLik	ΔAIC_c	Model weight
Senilia senilis				
t + s	7	1261.23	0.00*	0.27
s	**6**	**1259.66**	**0.96**	**0.17**
t + s + h + s:h	9	1262.47	1.97	0.10
t + s + h	8	1261.26	2.16	0.09
t + s + t:s	8	1261.25	2.17	0.09
s + h + s:h	8	1260.96	2.75	0.07
s + h	7	1259.68	3.10	0.06
t + s + h + t:h + s:h	10	1262.55	4.07	0.04
t + s + h + t:s + s:h	10	1262.53	4.11	0.03
t + s + h + t:h	9	1261.29	4.32	0.03
t + s + h + t:s	9	1261.27	4.36	0.03
t + s + h + t:s + t:h + s:h	11	1262.65	6.16	0.01
t + s + h + t:s + t:h	10	1261.31	6.53	0.01
*AIC_c = –2507.8				
Dosinia isocardia				
t + s + h	8	254.20	0.00*	0.22
t + s	**7**	**252.73**	**0.02**	**0.22**
t + s + h + t:s	9	255.18	1.13	0.12
t + s + t:s	8	253.63	1.14	0.12
t + s + h + s:h	9	254.84	1.80	0.09
t + s + h + t:h	9	254.47	2.54	0.06
t + s + h + t:s + s:h	10	255.60	3.52	0.04
s + h	10	255.44	3.84	0.03
t + s + h + t:s + t:h	7	250.68	4.13	0.03
t + s + h + t:h + s:h	10	255.10	4.52	0.02
s + h + s:h	8	251.60	5.21	0.02
s	6	248.72	5.27	0.02
*AIC_c = –488.61				
Loripes lucinalis				
t + s	**7**	**570.08**	**0.00***	**0.45**
t + s + h	8	570.21	2.08	0.16
t + s + t:s	8	570.20	2.10	0.16
t + s + h + s:h	9	570.33	4.19	0.05
t + s + h + t:s	9	570.31	4.24	0.05
t + s + h + t:h	9	570.30	4.24	0.05
t + s + h + t:s + s:h	10	570.42	6.43	0.02
t + s + h + t:s + t:h	10	570.40	6.46	0.02
t + s + h + t:h + s:h	10	570.39	6.47	0.02
*AIC_c = –1125.1				

(averaged over summer and winter) by 21.4% (95% CI of 8.5–34.2%; Table 9.2, Fig. 9.3B). For *Loripes*, *k* in winter was reduced by 34.6% (95% CI of 26.6–63.8%; Table 9.2, Fig. 9.3C) compared to summer calculated for control density treatments, while doubling the local bivalve density reduced *k* (averaged over summer and winter) for *Loripes* by 41.2% (95% CI of 24.7–66.9%; Table 9.2, Fig. 9.3C).

A sensitivity analysis with respect to the estimated value of H_∞ revealed that our results for *Dosinia*, *Loripes* did not change when increasing H_∞ (and the corresponding variance

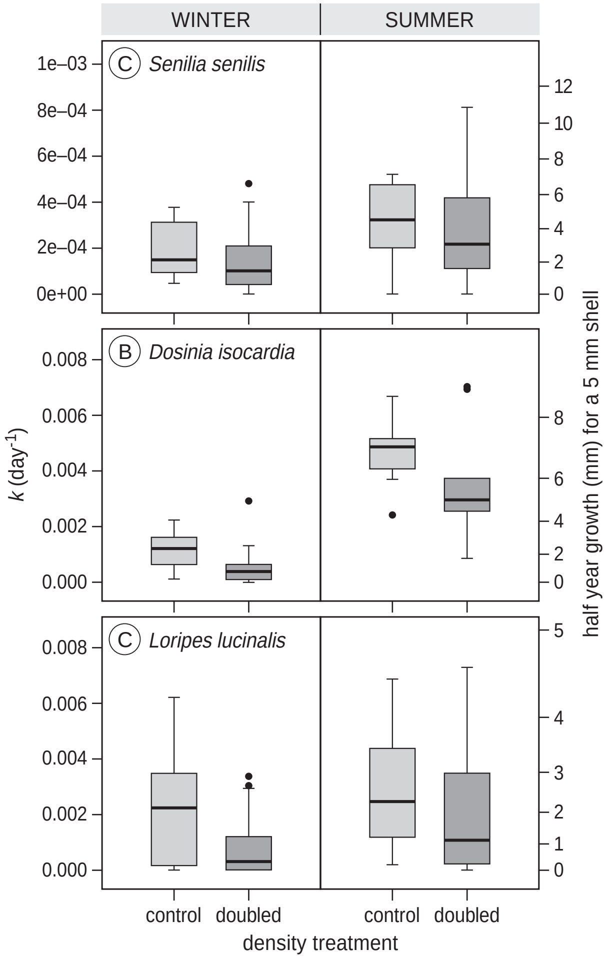


Figure 9.3 The effect of density treatment on growth constant *k* (day^{-1}) in both winter (i.e., October 2007–April 2008) and summer (April 2008–November 2008) for three different bivalve species (A) *Senilia senilis* (B) *Dosinia isocardia* (C) *Loripes lucinalis*. On the right y-axis *k* values are converted to half year (Δt = 182.5 days) growth (mm) for a shell with an initial shell height (H_1) of 5 mm by using equation (3) and the species-specific H_∞. Note that one outlier with a value of *k* (day^{-1}) = 0.013 is not presented for *Dosinia isocardia* (summer, control).

structure) across a range of values for H_∞ (*Dosinia*, 17.1–24 mm; *Loripes* 11.3–18 mm), reaching much beyond the natural range of H_∞ in these two species (respectively 17–20 and 10–12 mm; M. van der Geest and J. A. van Gils, *unpublished data*). For *Senilia* the result did not change when varying H_∞ (and the corresponding variance structure) between 77–80 mm. However, when setting H_∞ to values higher than 80 mm, the local density effect becomes significant. Given that >99.8% of the *Senilia* population has a shell height smaller than 80 mm ($N = 2{,}234$; M. van der Geest and J. A. van Gils, *unpublished data*), we believe that using our estimated value of $H_\infty = 77$ mm in our model analysis for *Senilia* is justified.

Table 9.2 Parameter estimates and approximate 95% confidence intervals for the most parsimonious model for growth constant k (day^{-1}; see Table 9.1) for *Senilia senilis*, *Dosinia isocardia* and *Loripes lucinalis*.

			95% C.I.	
		Estimate	Lower	Upper
Senilia senilis				
Intercept		0.000309	0.000233	0.000385
Season[1]	Winter	-0.000142	-0.000189	-0.000094
Random effects	$\sigma_{station}$	0.000078	0.000038	0.000160
	σ_{site}	0.000078	0.000053	0.000116
	$\sigma_{residual}$	0.000182	0.000151	0.000219
Variance function	$\sigma^2_{winter}/\sigma^2_{summer}$	0.436	0.327	0.582
Dosinia isocardia				
Intercept		0.00503	0.00495	0.00612
Season[1]	Winter	-0.00373	-0.00489	-0.00258
Treatment[2]	Density doubled	-0.00066	-0.00112	-0.00020
Random effects	$\sigma_{station}$	0.00004	0.00026	0.00194
	σ_{site}	0.00064	0.00039	0.00106
	$\sigma_{residual}$	0.00031	0.00017	0.00059
Variance function	$\sigma^2_{winter}/\sigma^2_{summer}$	0.134	0.064	0.271
Loripes lucinalis				
Intercept		0.00301	0.00208	0.00394
Season[1]	Winter	-0.00104	-0.00164	-0.00044
Treatment[2]	Density doubled	-0.00102	-0.00163	-0.00042
Random effects	$\sigma_{station}$	0.00087	0.00042	0.00180
	σ_{site}	0.00066	0.00036	0.00122
	$\sigma_{residual}$	0.00115	0.00090	0.00147
Variance function	$\sigma^2_{winter}/\sigma^2_{summer}$	0.521	0.717	0.986

Reference category: [1]summer, [2]control

Discussion

Feeding style-, season- and density-dependent growth

The lack of a significant effect of our density treatment on growth constant *k* in suspension-feeding *Senilia* is consistent with the predictions made by Levinton (1972) that benthic suspension-feeders are unable to deplete their food supply. That the best supported model for *Senilia* included an insignificant effect of density treatment, where doubled clam densities reduced *k* by 17.2% (95% CI of -2.8–37.2%; averaged over summer and winter), suggests that density-dependent growth may occur in *Senilia* at even higher densities than used in this study.

In contrast, we did find a significant effect of our density treatment on *k* in suspension-feeding *Dosinia*. Although belonging to the same feeding guild, *Senilia* and *Dosinia* are known to occupy different habitats, with *Senilia* living in bare sandy sediments and *Dosinia* living in more muddy seagrass-covered sediments (Honkoop *et al.* 2008; Fig. 9.2). If density-dependent growth in *Dosinia* is a result of resource limitation, and both bivalve species use the same resources (i.e., resuspended benthic diatoms), then it is tempting to conclude that resource availability for suspension feeders may have been habitat-dependent, with relatively low resource availability in seagrass-covered muddy sediments compared to bare sediments. Peterson *et al.* (1984) argued that the net effect of seagrass on growth of benthic suspension feeders may be set by the relative strength of two opposing factors, reduced current velocity, which promotes food depletion by suspension-feeding bivalves in the lower water column, and velocity deceleration, which enhances particle settlement towards the lower water column. If we follow this hydrodynamic hypothesis, the results from Honkoop *et al.* (2008), who found that the concentration of fresh (algal-derived) pigments from the top-5 mm sediment was smaller in seagrass-covered sediments than in bare sediment at Banc d'Arguin, suggest a seagrass system where food depletion at the bottom overrules enhanced particle settlement towards the bottom. Thus, habitat-dependent suspended food depletion may explain why density-dependent growth was only observed in suspension feeders living in muddy seagrass sediments (i.e., *Dosinia*).

Our study seems the first to reveal a significant density-dependent depression in growth in a chemosynthetically-fuelled bivalve *Loripes lucinalis* relying primary on sugars provided by sulphide-oxidizing gill-inhabiting bacteria for its nutrition (Johnson, Diouris & Le Pennec 1994; Chapter 2). This suggests that endosymbiotic bacterial chemosynthesis is limited by the transport of sulphide and/or oxygen to the gills by the bivalve host, which would imply that local rather than regional (e.g., whole coastal area of Banc d'Arguin) resource depletion is the mechanism by which resource limitation occurs in this symbiotic bivalve-bacteria association. Such a finding is in agreement with Childress and Griguis (2011), who showed that moderate to high rates of chemoautotrophic metabolism impose oxygen uptake demands upon the hosts that are much higher than is typical for the non-symbiotic annelid, bivalve and gastropod lineages to which they are related. As such, they suggested that the high oxygen demand of chemoautotrophic symbionts is perhaps the most limiting flux for the symbioses.

We observed a relatively large season effect on growth in all three bivalve species with reduced growth rates in the winter compared to the summer (Table 9.1 & 9.2, Fig. 9.3). This is consistent with previous studies at Banc d'Arguin investigating seasonality in growth rates of *Senilia* (Lavaud *et al.* 2013) and *Loripes* (Chapter 7). Interestingly, this effect of season on growth rate was relatively large for suspension-feeding *Dosinia* and *Senilia* compared to chemosymbiotic *Loripes* (a -76.0%, -45.8% and -36.0% growth reduction in winter compared to summer, respectively). This may indicate that, compared to chemosynthetically-fuelled *Loripes*, seasonality in 'phototrophic' food availability was more severe for suspension-feeding *Senilia* and *Dosinia*.

That we failed to demonstrate any significant habitat (i.e., PC1) effect on growth constant *k* in both suspension feeders and chemosymbiotic bivalves (Table 9.1), may be explained by the limited range of habitats occupied by recaptured individuals of each species (Fig. 9.2) making it less likely for such an habitat effect to be statistically detected. An alternative explanation is that our clams are distributed according to the ideal free distribution (IFD), which predicts that the number of individual animals will aggregate in various patches in proportion to the amount of resources available in each patch (Fretwell & Lucas 1970). If our focal clam species indeed follow an IFD, then habitat-specific natural densities of the benthic community will result in equal growth rates in bivalves among all habitats. Interestingly, the best supported model for *Dosinia* (but not the most parsimonious model) did include a habitat effect on *k* (Table 9.1). Running this model revealed that PC1, which increases with increasing seagrass cover and decreasing sediment grain size (see Appendix V, Fig. V.1), had an insignificant negative effect on *k* of *Dosinia* by 9% (95% CI of 0–19%; averaged over summer and winter and over both control and density treatment). Given that we hardly found any *Dosinia* in bare sediments where growth conditions seem more optimal, we argue that at least *Dosinia* does not have an IFD. This suboptimal distribution may be explained by higher predation pressure being exerted in bare sediments. Indeed, the dense rhizome mat created by the seagrass-related sediment structure is known to protect bivalves from encountering predators (e.g., Heck & Wetstone 1977; Peterson 1982; Irlandi & Peterson 1991).

Methodological considerations

Our experimental set-up allows bivalves to freely move away from our study plot. Hence, it is possible that at sites where ambient mollusc densities were doubled, emigration may have resulted in comparable densities within and outside the experimental plot at the time of recollection. Consequently, the observed treatment effects of ambient mollusc density manipulation are best viewed as minimal estimates of density-dependent growth.

Furthermore, the tagging procedure used in this study may have caused retarded bivalve growth due to stress. As an alternative to the use of glue-on-tags to mark clams, van der Geest *et al.* (2011) developed a non-invasive *in situ* calcein-marking method to determine growth rates in fragile burrowing bivalves (i.e., *Loripes lucinalis*). Using this calcein marking technique and $H_\infty = 11$ for *Loripes*, van Gils *et al.* (2012) reported a growth constant *k* (year^{-1}) = 0.66 for *Loripes* from our study site. Correcting this value for $H_\infty = 11.3$ as used in this study gives *k* (year^{-1}) = 0.63. For comparison, we have to correct this value for the time interval between marking and recollection of the marked specimen (i.e., one year), using the derivation of Fabens (1965) increment model (see equation (1) and (2)), which results in *k* (year^{-1}) = 0.99, this being equal to *k* (day^{-1}) = 0.00272. This value is only slightly higher (8%) than the mean annual *k* (day^{-1}) obtained in this study in control treatments (0.00249), suggesting that the effect of tagging on growth rate of *Loripes* (and presumably also on larger and less fragile *Dosinia* and *Senilia*) is limited.

Intra- versus interspecific competition

Although our field experiment provides important information on how close suspension-feeding and chemosymbiotic benthic populations at Banc d'Arguin are to carrying capacity, having manipulated densities of the total mollusc community, we were unable to separate effects of intraspecific from interspecific competition on growth in our three focal bivalve species. However, in view of the different feeding modes (suspension feeding vs. feeding mainly on chemosynthetically produced food) and the spatial segregation of the three numerically dominant bivalve species studied here (suspension feeding *Senilia* being restricted to bare sediments and suspension feeding *Dosinia* and chemosymbiotic *Loripes* mainly inhabiting seagrass sediments; Fig. 9.2), intraspecific competition seems of more importance than interspecific competition in the soft-sediment of Banc d'Arguin. However, exploitative competition for oxygen, which may be more severe in the anoxic organic-rich seagrass sediments compared to bare sediments, cannot be ruled out here (Ferguson, White & Marshall 2013).

Interactions with the main shorebird predators

Among shorebirds wintering at Banc d'Arguin, the red knot (Calidris canutus canutus) is the most abundant molluscivore (Altenburg *et al.* 1982). Based on their diets, and their energy requirements, knots should be responsible for ca. 80% of all mollusc consumption by vertebrate predators in Banc d'Arguin (Zwarts *et al.* 1998). Due to their relatively small size *Dosinia* and *Loripes* together make up 75% of all molluscs that are ingestible by red knots (Honkoop *et al.* 2008) and dominate the diet of red knots (Onrust *et al.* 2013; van Gils *et al.* 2013). In contrast, relatively large and thick-shelled *Senilia* does not experience high predation rates at Banc d'Arguin (Altenburg *et al.* 1982; Wolff *et al.* 1987). This species-specific difference in predation rate may explain why suspension-feeding *Senilia* can survive in bare sandy sediments where growth conditions seem better for suspension-feeders, while suspension-feeding *Dosinia* is restricted to seagrass sediments, where growth conditions are suboptimal, but predation rates are lower. Alternatively, competitive exclusion could play a role, with *Senilia* outcompeting *Dosinia* in bare sandy sediments. However, given the prevailing generalization that competitive exclusion plays a minor role in soft-sediments suspension-feeders communities (Peterson 1979; Wilson 1991; Peterson & Black 1993; and supported by the lack of density effects in suspension-feeding *Senilia* (this study)), we suggest this to be of less importance (but see Ferguson, White & Marshall 2013).

Currently, the population of wintering molluscivore red knots at Banc d'Arguin is in decline (van Gils *et al.* 2013). Given the observed density-dependent growth in their favourite *Dosinia* and *Loripes* prey, this decrease in predation pressure will increase competition for resources among their prey, which may result in a stunted reproduction-limited bivalve population due to overcompensation in stage-specific biomass production (de Roos *et al.* 2007). Subsequently, this could lead to a scenario referred to as the emergent Allee effect where population of red knots below a critical level collapses, resulting in a shift in prey population size-structure, ultimately leading to an alternative stable equilibrium in which the knots cannot return (de Roos, Persson & Thieme 2003; de Roos *et al.* 2007). We hope this scenario not to become reality.

Acknowledgements

We are deeply grateful to the Parc National du Banc d'Arguin (PNBA) for their permission to work in the park and for the use of their facilities. The PNBA crew at Iwik, and Lemhaba Ould Yarba at PNBA headquarters in Nouakchott are thanked for logistical support. We also thank the inhabitants of the PNBA Research Station in Iwik, especially Sidi Ould Ely and Sall Amadou Abderahmane for their cheerful contributions and co-operation. Annette van Koutrik is thanked for processing sediment samples. Tamar Lok, El Hacen Mohamed El Hacen, Marieke Smit, José Reinders and Erik Jansen assisted in collecting and processing benthic samples. Tamar Lok helped with constructive comments on statistics. We are grateful to Eelke Folmer for providing us with a shapefile of the habitats in our study area that we used to make Figure 9.1. This work was funded by the NWO-WOTRO Integrated Programme grant W.01.65.221.00 to T.P.

Appendix V

Section V.1

So far density-dependent growth (and survivorship) in bivalves had only been studied in infaunal suspension-feeding species using enclosures to maintain treatment densities of the focal species in the field (e.g., Peterson 1982; Peterson & Black 1987; Black & Peterson 1988; Peterson & Beal 1989; Peterson & Black 1993). Generally, in these studies, enclosures are placed after which all living infaunal organisms are removed from the enclosed plot, to which variable densities of the focal bivalve species, all being individually measured and marked, are added. Subsequently, bivalve growth and/or survivorship over time are measured per treatment. Although such enclosure experiments provide a wealth of quantitative data on density-specific processes in soft-sediment suspension-feeding bivalves, there is also widespread concern about enclosure (and exclosure) experiments, because enclosure walls baffle currents, potentially altering sedimentation rates and the supply of suspended particulate organic matter, which often result in unnatural growth rates in the target species (Peterson & Beal 1989; Wilson 1991; Peterson & Black 1993). Furthermore, excavation of all macrofauna from the experimental plot, before relocation of known densities of marked individuals of the target species to the enclosed plot, destroys the surface structure of the sediments (especially in seagrass-covered sediments), which may also have significant effects on growth rate and/or other individual, population and community parameters. This effect may even be stronger in benthic soft-sediment organisms belonging to feeding guilds that in contrast to suspension feeders obtain their food from the surrounding sediment and/or pore-water (e.g., deposit feeders and chemosymbiotic organisms). Given the artefacts that may be imposed by the use of enclosures in a high energy environment like the intertidal Banc d'Arguin, our study area being dominated by seagrass beds, and the knowledge that one of our three focal species has a chemosynthetically-fuelled diet, we used an alternative experimental set-up to manipulate clam densities while keeping growing conditions as natural as possible, as described in the section *Methods–Experimental design*.

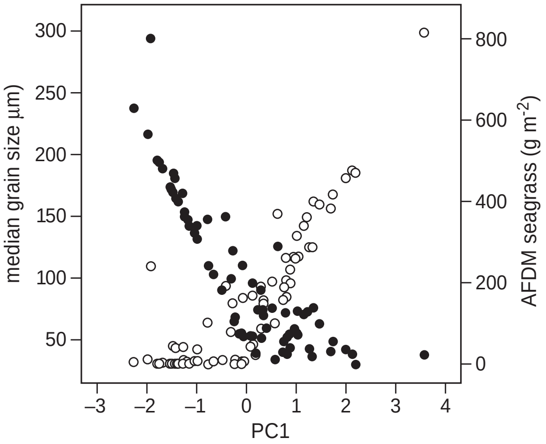


Figure V.1 Relationship between results of the first principle component (PC1) and median grain size of the sediment (closed circles) and the ash-free dry mass (AFDM) of all living above- and below-ground parts of seagrass *Zostera noltii* (open circles). Each data point represents the value for a site ($N = 70$).

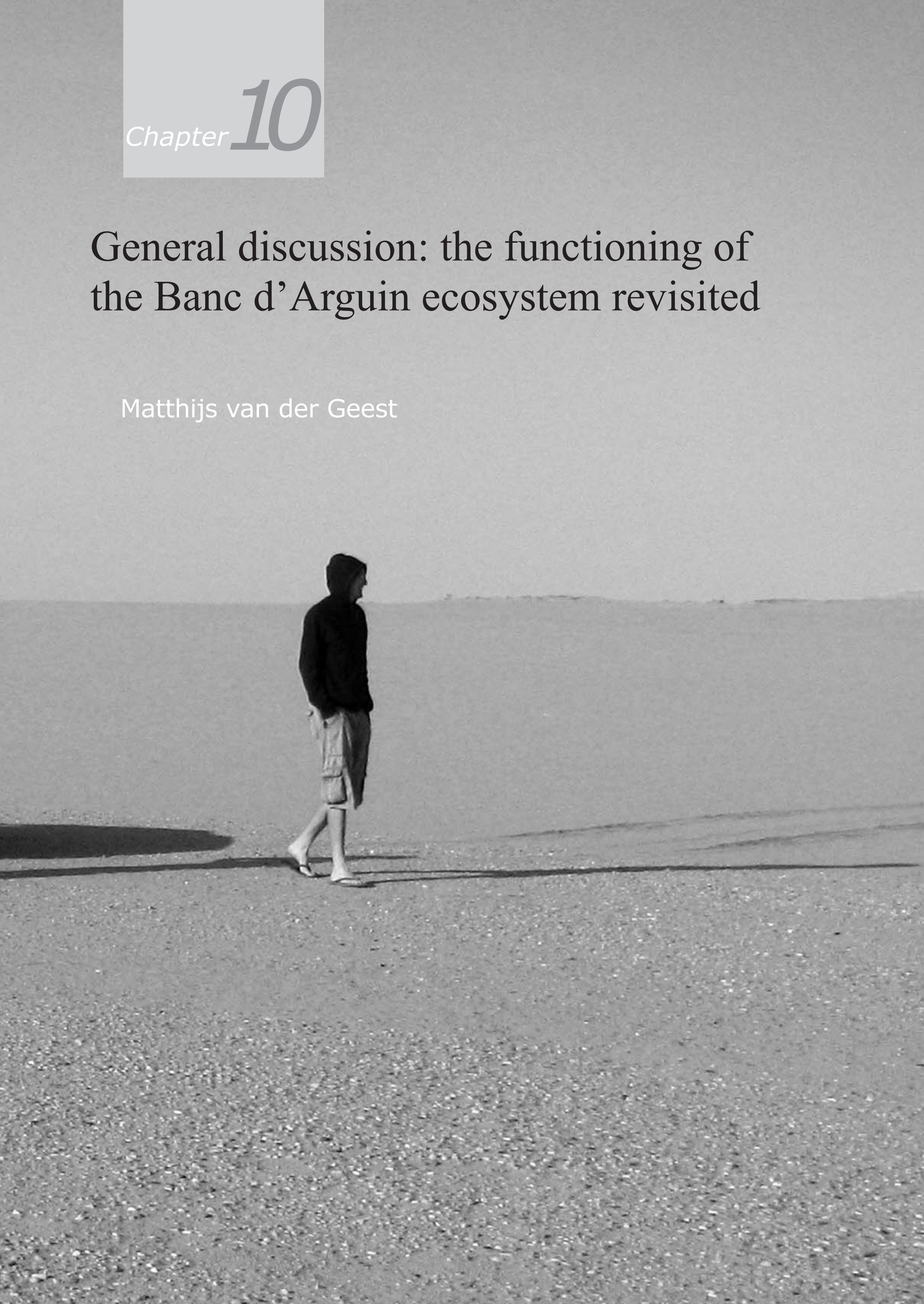
Table V.1 Fate of clams for three bivalve species over two time periods as a function of density treatment. Winter = October 2007–April 2008, Summer = April 2008–November 2008, N = the number of tagged clams used, H_1 = mean initial shell height (mm), A = percentage recaptured alive, D = percentage recaptured dead, M = percentage missing, U = percentage of clams of which the fate was unknown.

Species	Season	Treatment	N	H_1 (± SD)	Percentage recaptured			
					A	D	M	U
Senilia senilis	Winter	Control	68	17.9 ± 12.2	31	22	47	0
		Doubled	199	31.3 ± 17.3	35	20	44	2
	Summer	Control	78	25.3 ± 13.7	15	26	55	4
		Doubled	196	31.1 ± 16.1	35	16	47	2
Dosinia isocardia	Winter	Control	49	7.9 ± 3.4	22	16	16	45
		Doubled	160	9.1 ± 3.8	10	11	63	16
	Summer	Control	62	9.7 ± 3.8	15	8	37	40
		Doubled	97	9.7 ± 3.9	11	24	35	30
Loripes lucinalis	Winter	Control	78	6.6 ± 1.5	22	26	41	12
		Doubled	192	7.3 ± 1.6	18	18	57	7
	Summer	Control	84	6.6 ± 1.3	25	10	5	61
		Doubled	210	7.2 ± 1.6	20	14	38	28
		Total	1,473					

Chapter 10

General discussion: the functioning of the Banc d’Arguin ecosystem revisited

Matthijs van der Geest



This thesis addresses a long standing enigma of an apparent mismatch between prey biomass and predator consumption on the tropical seagrass-covered mudflats of Banc d'Arguin, Mauritania, supporting vast numbers of migratory shorebirds (Altenburg *et al.* 1982; Wolff & Smit 1990; Zwarts *et al.* 1990; Michaelis & Wolff 2001). Low standing stocks of macrobenthic shorebird food implies high secondary productivity (Piersma 1982), but without a trace of the offshore oceanic upwelling (Sevrin-Reyssac 1984, 1993), and seagrass considered too refractory (Honkoop *et al.* 2008), the carbon source of this secondary production remained unaccounted for (Wolff *et al.* 1993b). This thesis aimed to elucidate the role of endosymbiotic chemoautotrophy in explaining the productivity of these intertidal seagrass habitats, a metabolic pathway hitherto not fully considered.

The main objectives of the present thesis are to improve our understanding of the flux of chemosymbiotically fixed carbon within this seagrass-covered intertidal ecosystem, to reveal the organizing principles (predation, competition, mutualism) that mould this seagrass ecosystem. Of course we also like to see that sound knowledge can then be used for effective management of this exceptional coastal area. These objectives are addressed using the intertidal seagrass *Zostera noltii*, the chemoautotrophic endosymbiont-bearing bivalve *Loripes lucinalis* and a migratory molluscivore shorebird, the red knot *Calidris canutus canutus*, as model species. This final chapter integrates the most important findings in this thesis with existing knowledge on the functioning of Banc d'Arguin and other intertidal seagrass ecosystems. First, I will discuss how chemoautotrophic associations contribute towards the carrying capacity of the Banc d'Arguin ecosystem, to come to a new model of the carbon fluxes within this tropical seagrass ecosystem in which the chemoautotrophic metabolic pathway is incorporated. Next, I will synthesize results from previous chapters to come to secondary production rates for the predominant bivalve populations (i.e., *Loripes lucinalis*, *Dosinia isocardia* and *Senilia senilis*) and discuss if these production rates are sufficiently high to support the large numbers of molluscivore red knots wintering at Banc d'Arguin. I will continue by discussing which biotic and abiotic factors structure the carbon fluxes within this seagrass system, to end with a special focus on the role of top-down regulation of these carbon fluxes by avian predators. Finally, implications of the presented findings for tropical seagrass ecosystem management are put forward and future research directions are suggested.

The Banc d'Arguin carbon flow revisited

After the vast numbers of birds at Banc d'Arguin were first discovered, it was assumed that this abundance of secondary consumers would be due to the existence of an oceanic 'upwelling' along the coast of Mauritania, causing primary productivity by phytoplankton to be exceptionally high and the trophically dependent species numerous (De Naurois 1959), a rather impressionistic view that has persisted until quite recently (Butler, Davidson & Morrison 2001). However, Sevrin-Reyssac (1984; 1993) and Wolff *et al.* (1993b) concluded that the upwelling of the Mauritanian coast exhibits no influence on the tidal ecosystem of Banc d'Arguin. They suggest that primary producers other than phytoplankton must play an important role in the energy flow of this ecosystem. Low abundance of benthic macroalgae and mangroves (*Avicennia africana*) at Banc d'Arguin make these primary producers of limited importance (Wolff & Smit 1990; Wolff *et al.* 1993b). Instead, approximately 80% of the intertidal area of Banc d'Arguin is partially or completely covered by seagrasses (Wolff & Smit 1990). Although seagrass meadows are very productive and generally have large standing stocks of organic matter (i.e., seagrass material, Duarte & Chiscano 1999), direct grazing on seagrass products by benthic invertebrates is limited (Mateo *et al.* 2006). This is attributed to the presence of relatively high amounts of structural cell wall compounds and toxic or inhibitory chemicals such as phenolic compounds and polysaccharides (Godshalk & Wetzel 1978; Thayer *et al.* 1984).

However, the presence of seagrass is known to enhance sediment organic matter content by increasing sedimentation through a reduction of current velocity (Fonseca & Fisher 1986) and by *in situ* degradation of seagrass material. Hemminga & Nieuwenhuize (1991) indeed observed that the major part of the seagrass leaves at Banc d'Arguin remains trapped in the seagrass beds and decomposes *in situ*. With a large part of the macrozoobenthic fauna at Banc d'Arguin considered to be deposit-feeding, Wolff *et al.* (1993b) concluded that the intertidal Banc d'Arguin ecosystem is dominated by a detritus-based benthic food web in which seagrasses are the principal primary producers.

In this thesis we provide both molecular (box A) and stable-isotopic (Chapter 2) evidence that the lucinid bivalve species *Loripes lucinalis* (hereafter *Loripes*), which is numerically the most dominant mollusk in the seagrass sediments of Banc d'Arguin (Honkoop *et al.* 2008), largely feeds on carbon metabolites provided by endosymbiotic chemoautotrophic sulphide-oxidizing bacteria. We also show that this chemosynthetically fixed carbon is transferred to higher trophic levels because on average *Loripes* represents about 50% of the mollusk diet of red knots (see Fig. 8.5). Furthermore, the bi-annual release of gametes by *Loripes* (Chapter 2) forms another trophic link between chemosynthetically fixed carbon and the benthic (i.e., encapsulated eggs that adhere together on the bottom of the seagrass bed) and pelagic (i.e., free-swimming sperm cells) compartment. With densities up to 5,000 individuals m^{-2} (Chapter 4), *Loripes* is the most important chemosymbiont-bearing bivalve at the seagrass-covered tidal flats of Banc d'Arguin. Further research is needed to reveal the role of other benthic invertebrates (e.g., polychaetes, crustaceans, and other bivalves) potentially living in symbiosis with sulphide-oxidizing bacteria in the reduced seagrass sediment.

In this thesis we have provided strong support for the presence of an important chemotrophically fixed carbon flux within the seagrass beds of Banc d'Arguin that was hitherto not accounted for. Based on the detritus-based food chain picture (Fig. 10.1A) presented by Wolff *et al.* (1993b) we have constructed a new conceptional model of the functioning of this seagrass-dominated intertidal 'chemosynthetic' ecosystem as presented in Fig. 10.1B.

Whereas Wolff *et al.* (1993b) considered seagrasses to be the dominant primary producer at Banc d'Arguin, accumulating evidence shows that sediment microalgae (i.e.,

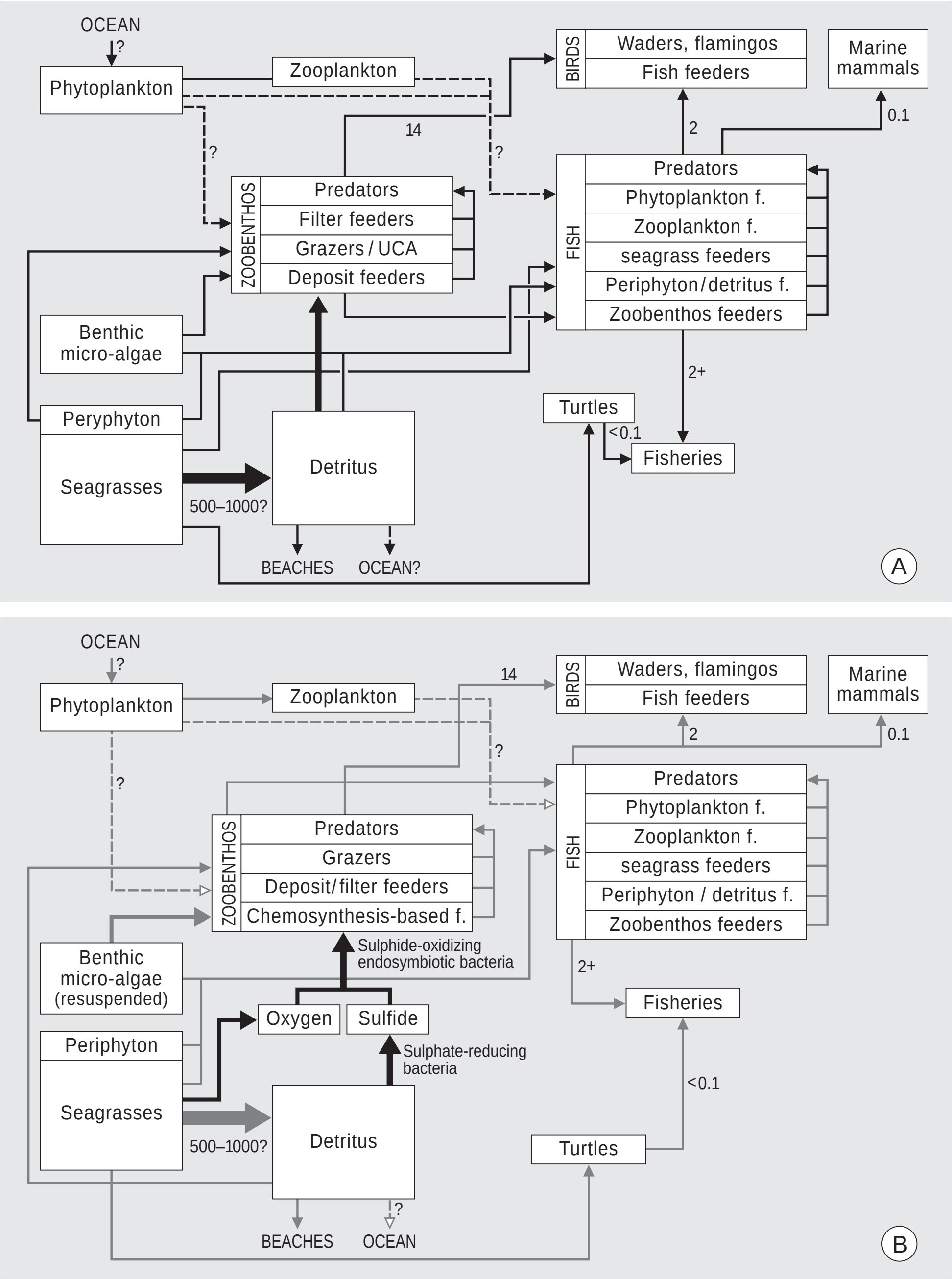


Figure 10.1 (A) The original (Wolff *et al.* 1993b) and (B) the revised conceptual models of carbon flux within the seagrass-covered intertidal ecosystem of Banc d'Arguin. Black arrows in panel B denote the novel chemoautotrophic metabolic pathway. The thickness of the arrows denotes the estimated size of the transport rates; in some cases a preliminary quantification (as g AFDM m^{-2} y^{-1}) has been added for an area of about 1,000 km^2 of shallow water and tidal flats around the island of Tidra.

microphytobenthos) can also exhibit high production rates in seagrass beds (Asmus & Asmus 1985; Kaldy *et al.* 2002, Mateo *et al.* 2006), sometimes yielding up to 54% of the primary production in seagrass beds (Lebreton *et al.* 2009). A recent study by Honkoop *et al.* (2008) in Banc d'Arguin indeed showed that concentrations of benthic diatom-produced phytopigments in both seagrass covered and bare sediment were similar to those observed at the nutrient-rich mudflats of the Dutch Wadden Sea. Evidence for a benthic diatom-based food web also comes from stable-isotopic analysis of predominant primary and secondary producers at the intertidal flat area surrounding the Iwik peninsula, Banc d'Arguin, with mean $\delta^{13}C$ values of -13.7‰ (± 1.7 SD, $N = 8$) for benthic diatoms and -18.1‰ (± 1.6 SD, $N = 4$) for phytoplankton sampled in April–May 2008 and May 2008, respectively (M. van der Geest & M. Smit, *unpublished data*). Accordingly, the mean $\delta^{13}C$ value of -15.5‰ obtained for small suspension feeding *Dosinia isocardia* (Chapter 2), accounting for a trophic enrichment of ~1‰ (Peterson & Fry 1987), can be considered to reflect a dietary carbon dominated by (resuspended) microphytobenthic diatoms. In light of these results, we tentatively enlarged the arrow from benthic microalgae towards zoobenthos in our new conceptional model of the functioning of the intertidal Banc d'Arguin ecosystem (Fig. 10.1B). Yet, the role of microphytobenthos in the Banc d'Arguin seagrass bed food web, notably the contribution of microphytobenthos as a food resource to macrobenthic fauna, is poorly understood and thus provides an interesting topic for future research.

Benthic production and consumption rates at Banc d'Arguin

As stated in the General introduction, the enigmatic situation that over two million wintering shorebirds depend on low standing stocks of macrozoobenthic food in all intertidal habitats of Banc d'Arguin, implied sufficiently high production rates of the benthic fauna (Piersma 1982; Wolff 1991; Wolff *et al.* 1993b; Michaelis & Wolff 2001), but these are unaccounted for.

Wolff and Smit (1990) propose that the annual disappearance of benthic biomass due to predators should be at least 20 g ash-free dry mass (AFDM) m^{-2}. When taking the mean value of the macrozoobenthic biomass (excluding the inedible *Senilia senilis*) reported in previous benthic surveys that covered at least 40% of the entire Banc d'Arguin tidal flat area (see Table 1.1), this gives a value of 6.9 (± 2.7 SD) g AFDM m^{-2}. Thus, a minimal annual production/average biomass ratio (P/B ratio) for the benthic fauna of around 3 is needed to supply the estimated energetic demands of all macrozoobenthic predators. So far, the only zoobenthic P/B ratio estimated at Banc d'Arguin is provided by Wolff *et al.* (1987), who arrived at a value of 0.02 yr^{-1} for a *Senilia* population consisting of large (>60 mm) and very old (>14 years) specimens. Other fauna largely consists of smaller species that are likely to have higher P/B ratios (Piersma 1982; Wolff & Smit 1990), but estimates are lacking.

Here we take up this challenge by combining our results from the benthic survey described in Chapter 6 with bivalve growth estimates presented in Chapter 9 to quantify production rates and P/B ratios for two relatively small but numerically abundant bivalve species, *Loripes lucinalis* and *Dosinia isocardia* (hereafter *Dosinia*).

We will use the estimated P/B ratios to test whether production rates of these two staple prey species are sufficient to sustain the molluscivore red knot population at Banc d'Arguin. Furthermore, comparison of the species specific P/B ratios enables us to test the hypothesis stated in the General introduction that in the clear oligotrophic waters of Banc d'Arguin, chemosymbiotically fuelled *Loripes* would have a higher P/B ratio than suspension feeding *Dosinia*.

Despite its low productivity of 0.01 g AFDM m^{-2} yr^{-1} measured in 1985–1986 (Wolff *et al.* 1987), the standing stock of *Senilia senilis* (hereafter *Senilia*) has increased tremendously over the last two decades (from 0.8 to 20.3 g AFDM m^{-2} yr^{-1}; Table 1.1). Honkoop *et al.* (2008) suggested that this increase in *Senilia* biomass densities may indicate better feeding conditions for this suspension feeder. This would imply that production rates are currently higher than in the 1980s. To test this hypothesis, we also use data presented in Chapter 6 and 9 to quantify production rates of *Senilia*.

As summarized by van der Meer *et al.* (2013), the production of a marine bivalve population can be estimated by means of relatively simple methods, such as the *increment-summation* method for populations with identifiable cohorts. Basically, the advantage of such cohort data is that they enable the estimation of both the survival function and the growth function, which are essential ingredients of production estimates. In the temperate zone, bivalve cohorts can often be separated on the basis of annual growth rings, and in some of the tropical Banc d'Arguin species such growth rings can be found too (e.g., *Senilia*, Wolff *et al.* 1987; van der Geest *et al.* 2010). For populations that do not produce identifiable cohorts (and this includes *Loripes* and *Dosinia* at Banc d'Arguin), production rate at any point in time can be calculated directly from the size-frequency distribution by the so-called mass-specific analogue of the *increment-summation* method, provided that the size-mass relationship and the growth function is known (Winberg 1971; van der Meer *et al.* 2013).

Here we use the data from the full-year benthic survey (March 2011–February 2012) carried out at the tidal flat area Abelgh Eiznaya, near Iwik (Chapter 6) to obtain monthly size-frequency distributions of *Loripes*, *Dosinia* and *Senilia* (Figs. 10.2–4). Subsequently, we use the season-specific Von Bertalanffy growth function parameters obtained in Chapter 9 (summarized in Table 10.1), together with the ash-free dry mass (AFDM, in grams)-to-

Table 10.1 Season-specific growth parameters used in Von Bertalanffy growth function for three bivalve species from Banc d'Arguin, Mauritania (for more details see Chapter 9). H_∞ denotes maximum shell height (mm).

Species	k (day^{-1}) in November–April	k (day^{-1}) in May–October	H_∞
Loripes lucinalis	0.00197	0.00301	11.3
Dosinia isocardia	0.0013	0.00503	17.1
Senilia senilis	0.000167	0.000309	77.0

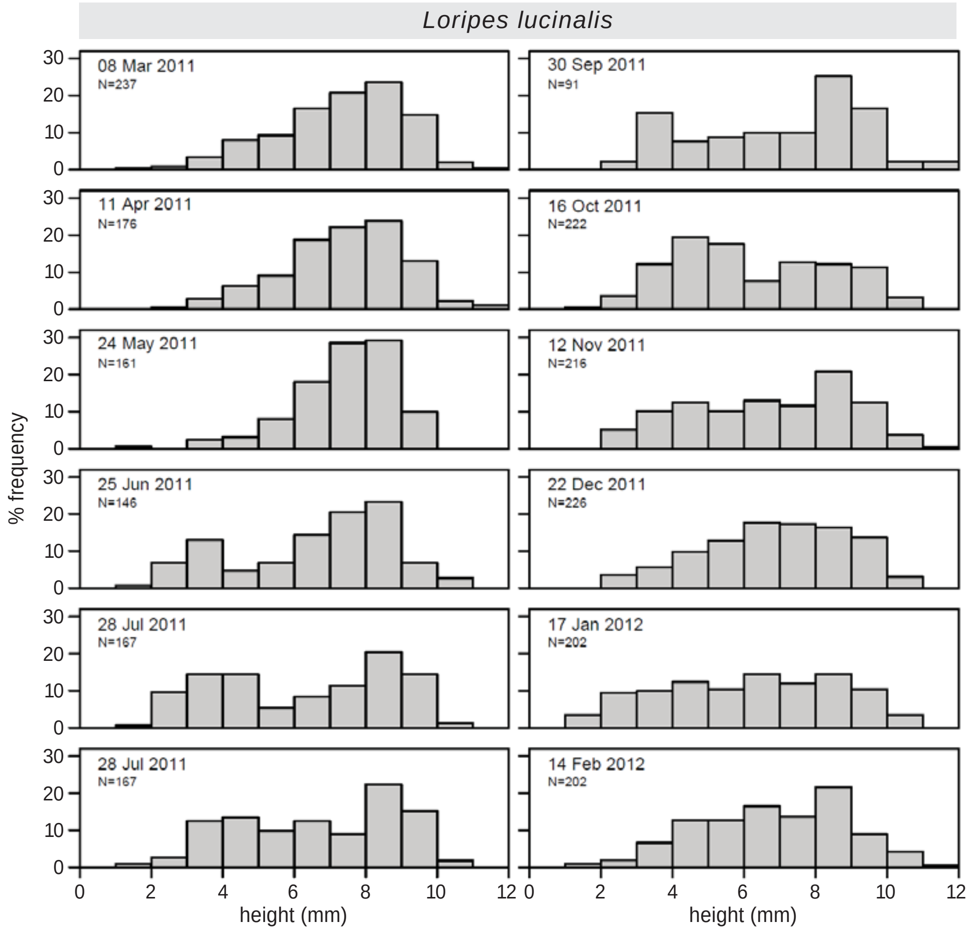


Figure 10.2 Size-frequency distribution of *Loripes lucinalis* from at tidal flats of Abelgh Eiznaya, Banc d'Arguin, Mauritania, from March 2011–February 2012 (dataset Mohamed V. A. Salem *et al.*, Chapter 6).

shell height (H, in millimeters) relationships (*Loripes*, ln(AFDM) = –10.27 + 2.74 × ln(H); *Dosinia*, ln(AFDM) = –10.83 + 2.75 × ln(H); *Senilia*, ln(AFDM) = –10.46 + 2.66 × ln(H)), to calculate species-specific production rates per time interval as described by Winberg (1971) and van der Meer *et al.* (2013). Thus, we used the size-frequency distribution at a given sampling date t_2 and the season-specific growth function to predict the size-frequency distribution at the intermediate date between sampling date t_2 and sampling date t_1 ($t_{1.5}$) and between sampling date t_2 and sampling date t_3 ($t_{2.5}$). The size-mass relationship can now be used to predict the standing biomass at $t_{1.5}$ and $t_{2.5}$ and the production for this time interval can be derived by subtracting the predicted biomass at $t_{1.5}$ from that predicted at $t_{2.5}$. Summing up the estimated production for all monthly intervals gives the total production for the given period, covering 11 months. By assuming that the size-frequency distribution

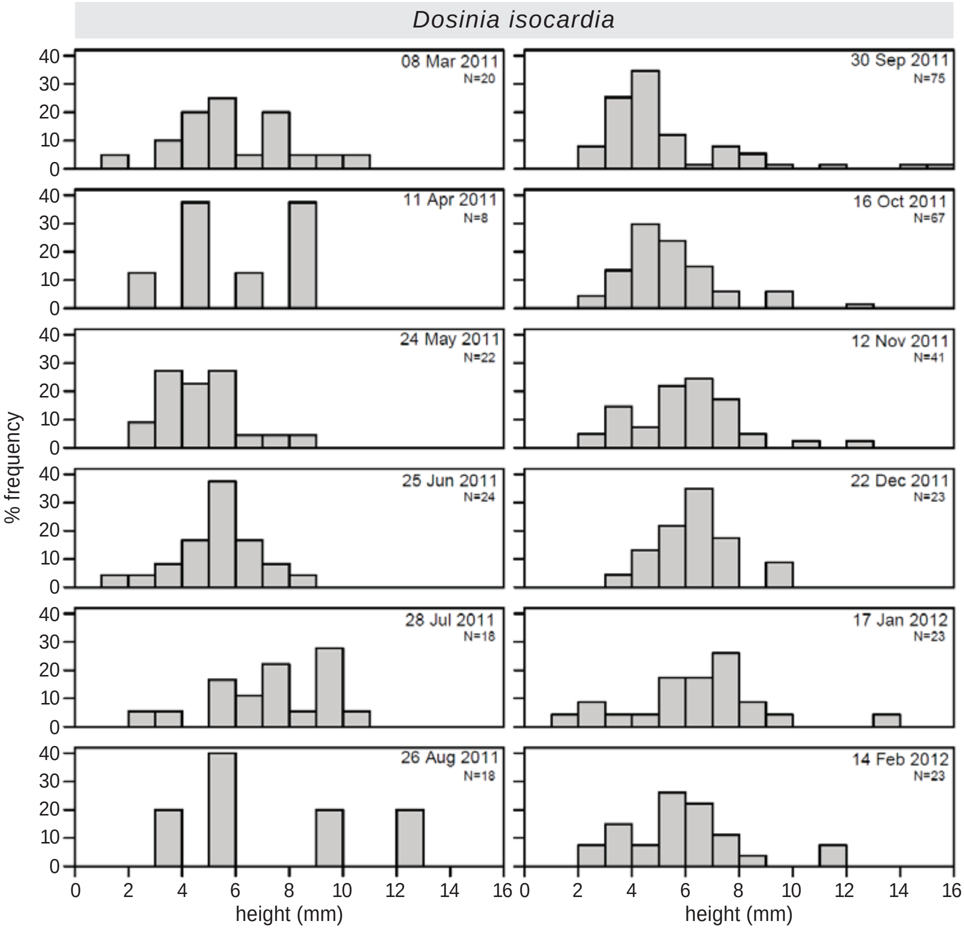


Figure 10.3 Size-frequency distribution of *Dosinia isocardia* at the tidal flats of Abelgh Eiznaya, Banc d'Arguin, Mauritania, from March 2011–February 2012 (dataset Mohamed V. A. Salem *et al.*, Chapter 6).

in March 2012 (not sampled) was the same as that in March 2011 (sampled), we were able to estimate the production in the final (12th) interval, using the size-frequency distributions in February 2012 and March 2011 to predict the standing biomass at $t_{11.5}$ and $t_{12.5}$ (= $t_{0.5}$) respectively.

The calculations resulted in production rate estimates of 5.40, 1.40 and 4.09 g AFDM m^{-2} yr^{-1} for *Loripes*, *Dosinia* and *Senilia*, respectively. Using mean annual standing stocks of 5.15, 0.34 and 24.09 g AFDM m^{-2} for *Loripes*, *Dosinia* and *Senilia* (see also Chapter 6), we obtained P/B ratios of 1.06, 4.1 and 0.17 yr^{-1} for *Loripes*, *Dosinia* and *Senilia*, respectively.

With P/B ratios of 1 for *Loripes* and 4 for *Dosinia*, our hypothesis that chemosymbiotic-fuelled bivalves such as *Loripes* would have higher P/B ratios than suspension feeding bivalves such as *Dosinia* is therefore rejected. Apparently, a 'photosynthetic' diet based on suspended particulate organic matter (POM) is a better substrate for growth than a 'chemosynthetic' diet. This may explain why *Loripes* additionally feeds on suspended POM, which at times can make up 24% of its diet (as demonstrated in our carbon stable isotope study presented in Chapter 2). Furthermore, results obtained from our exclosure experiment (Chapter 5) suggest that in the exclosure, without predation on *Dosinia*, stronger competition for suspended POM forces *Loripes* to rely on energy produced by endosymbiotic sulphide-oxidizing bacteria, thus leading to an enhanced uptake of sulphide from the surrounding pore-water, which was reflected by lower sulphide concentrations in the exclosures. The lower growth rates of *Loripes* within the exclosure then are consistent with the idea that, per unit time, energy obtained from chemosymbiontically fixed carbon is low compared with energy obtained from suspended POM.

Compared with the production rate of 0.01 g AFDM m^{-2} yr^{-1} and P/B ratio of 0.02 yr^{-1} estimated in 1985–1986 by Wolff *et al.* (1987), suspension-feeding *Senilia* have increased production rate by a factor 400, and annual turn-over by a factor 8.5, but still their P/B ratio and production rate are low compared with *Loripes* and *Dosinia*. This appears related to their different life history strategies, with *Senilia* becoming much larger and older than *Loripes* and *Dosinia*. Because of this, the annual production of *Senilia* is smaller than their average biomass, while that of smaller *Loripes* and *Dosinia* is larger than their average biomass.

The increased productivity of the *Senilia* population can be attributed to the major difference in size-structure of the *Senilia* population between the two periods, with the *Senilia* population in the 1980's being dominated by specimen >60 mm in shell height (Wolff *et al.* 1987), and the current population being dominated by relatively small specimens measuring 0–30 mm in shell height (Fig. 10.4). The more successful recruitment of suspension-feeding *Senilia* at present suggests that suspended particulate organic food conditions have ameliorated.

Loripes and *Dosinia* together represent 80% of the mollusks consumed by red knots at Banc d'Arguin (Onrust *et al.* 2013; Chapter 5, 8). Assuming that red knots are the only predators feeding on *Loripes* and *Dosinia*, the production rates by *Loripes* and *Dosinia* together must thus be at least 80% of the consumption rates by red knots to sustain both prey and predator populations. To examine the degree of fit, we will compare estimates of consumption with estimates of production for the Abelgh Eiznaya area.

At Abelgh Eiznaya, the average red knot feeding density of 18.3 birds ha^{-1} was derived from unpublished observations of feeding red knots carried out in March 2007 and February 2008 (J. A. van Gils, M. van der Geest, B. De Meulenaer, H. Gilles, T. Piersma & E. O. Folmer, *unpublished data*). Unfortunately, we have no detailed counts available during the period of monthly benthos sampling (March 2011–February 2012), but the fact that we did not find a significant difference between the 2007 and the 2008 counts suggests that inter-annual variations in red knot numbers will not affect the robustness of the present reconstruction. Note that Leyrer *et al.* (2006; 2012) found that red knots wintering at Banc d'Arguin show a high degree of site fidelity within and between years, using a 2–16 km^2

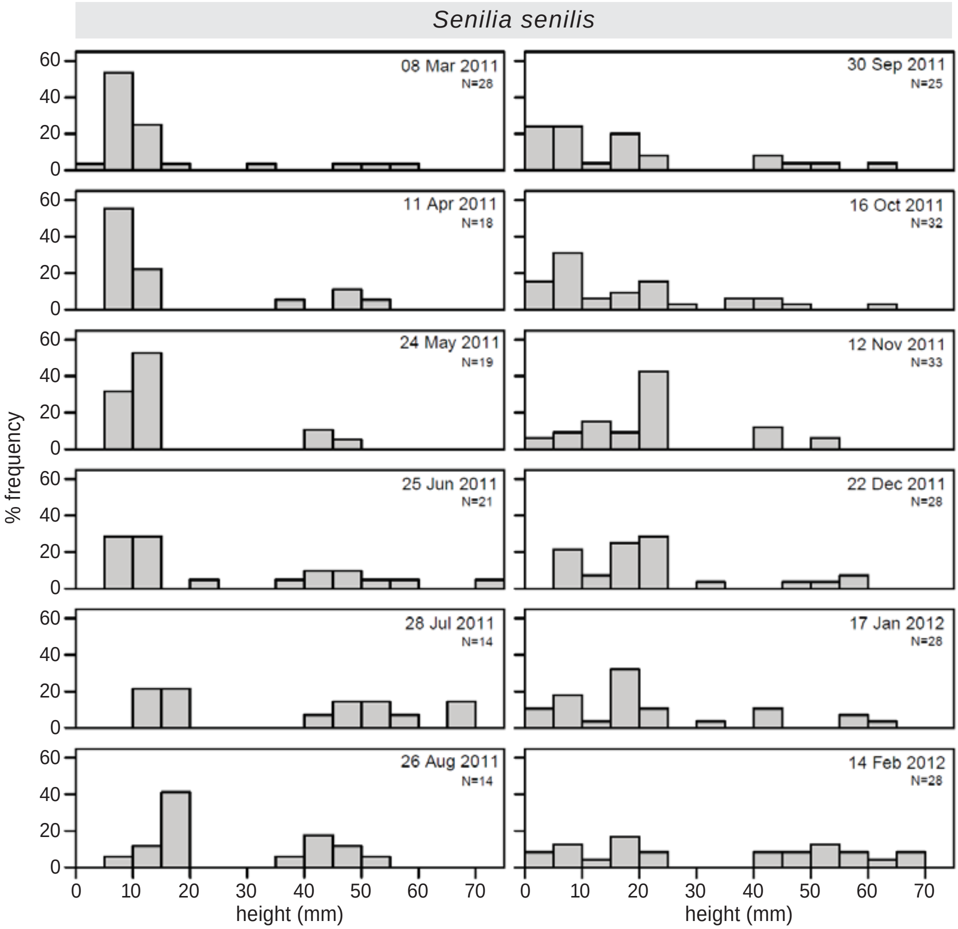


Figure 10.4 Size-frequency distribution of *Senilia senilis* at the tidal flats of Abelgh Eiznaya, Banc d'Arguin, Mauritania, from March 2011–February 2012 (dataset Mohamed V. A. Salem *et al.*, Chapter 6).

intertidal area only. Given that only 7% of the red knot population stays at Banc d'Arguin in summer, between May and August (van Dijk *et al.* 1990), we arrive at a mean annual red knot density at Abelgh Eiznaya of 12.5 individuals ha^{-1}. To maintain a balanced energy budget, red knots feeding at Banc d'Arguin require a minimum intake rate of 0.2 mg AFDM s^{-1} during 12 hours of feeding per day (van Gils *et al.* 2009). Given their densities this suggests an annual consumption of 3.94 g AFDM m^{-2} yr^{-1} by the total red knot population. *Loripes* and *Dosinia* should thus produce a minimum of 3.16 g AFDM g m^{-2} yr^{-1} to sustain both the local prey and predator populations.

At first sight, the estimated production rates of 5.40 and 1.40 g AFDM m^{-2} yr^{-1} for *Loripes* and *Dosinia*, respectively, may seem sufficient. However, not all of the produced biomass by *Loripes* and *Dosinia* is directly available, as knots are gape-limited to *Dosinia*

<13.2 mm and bivalves burrowed deeper than 4 cm are out of their reach (red knots have bills ~3.5 cm long). Moreover, in Chapter 8 we showed that intake rates on *Loripes* by red knots are limited to a maximum of 0.1 mg AFDM s^{-1}, because *Loripes* causes diarrhoea when consumed in great quantity (Oudman *et al.* in revision; Chapter 5, 8). This implies that *Loripes* can 'only' account for 1.58 g AFDM m^{-2} yr^{-1} of the required 3.16 g AFDM m^{-2} yr^{-1} by knots, still being 30% of its estimated total annual production in 2011–2012. The remaining 1.58 g AFDM g m^{-2} required by the local red knot population must thus be provided by *Dosinia*, a value that is more than 90% of the total annual production and average annual standing stock together (0.34 + 1.40 = 1.74 g AFDM m^{-2} yr^{-1}). Because this predation is mainly exerted in the winter period, it may come as no surprise that we observed *Dosinia* biomass densities at Abelgh Eiznaya to decline in the course of winter (Chapter 6 & Fig. 10.2).

A recent study by Leyrer *et al.* (2013) revealed that within the annual cycle of red knots, most mortality occurred during their stay at the tropical wintering grounds of Banc d'Arguin. This mortality could be caused by a lack of available *Dosinia* in some years because variation in annual apparent survival was positively correlated with *Dosinia* biomass density (Chapter 8). This highlights the importance of a good understanding of the life-history of *Dosinia* and the mechanisms that regulate their population dynamics, as well as the potential interactions between *Dosinia* and *Loripes* (as suggested in Chapter 5), knowledge which is currently lacking. Whether the strong evidence for food limitation in red knots is a more general phenomenon for the other shorebirds wintering at Banc d'Arguin remains a topic for further research.

Multi-trophic interactions in the Banc d'Arguin seagrass community

According to Valentine and Duffy (2006), the principal open question for seagrass ecology remains the understanding of the relative importance of bottom-up versus top-down control. What are the relative importances of resource supply, consumer pressure, and –perhaps most importantly– their interactions, in regulating the performance and persistence of seagrass beds? Although Valentine and Duffy (2006) refer to the central effects of grazing in seagrass ecology, we reveal the importance of positive feed-back mechanisms (Chapter 3). We show that a three-stage symbiosis between seagrass, lucinid bivalves and their sulphide-oxidizing bacteria reduces sulphide stress for seagrass, a mechanism that we propose to explain the long-term success of seagrasses in tropical waters (Chapter 3). The inference that symbioses rather than a single defining species form the foundation of seagrass ecosystems offers new prospects for seagrass ecosystem conservation.

That the seagrass community is also regulated by a top-down mechanism is supported by our predator-exclosure experiment (Chapter 5). This demonstrated a three-level trophic cascade induced by predation by molluscivore red knots affecting pore-water sulphide concentration via competitive release of the prey. On a short time scale, the exclusion of red knots works out negatively for *Loripes*, as reflected by reduced rates of shell growth, but may be beneficial for other organisms as it reduces pore-water concentrations of toxic sulphide (Chapter 5). However, in the absence of predation, the lower growth rates of *Loripes* may lead to lower reproduction in *Loripes*, eventually leading to a *Loripes* population decline (Persson *et al.* 2007). This will likely have the opposite effect on pore-water biogeochemistry, leading to increased levels of sulphide since there would be no *Loripes* present to keep sulphide concentrations low as experimentally demonstrated in Chapter 3. This would hamper most organisms living in the seagrass-covered tidal flats, including the seagrass itself (Chapter 3).

Additional support for top-down regulation of the seagrass community is provided in Chapter 7. Here we show that great variability in the 'date-of-birth-dependent offspring predation rate' may have moulded timing of reproduction in *Loripes*. It would be very interesting to also test this hypothesis for *Dosinia*, which per capita may experience even higher predation rates than the most heavily predated intermediate size-classes of *Loripes* (see Fig. 7.1). Top-down regulation of the seagrass community, may also be indicated by our observation that both *Loripes* and *Dosinia* show density-dependent growth at experimentally increased field densities (Chapter 9). Again, this supports our believe that by thinning prey, molluscivore predators at Banc d'Arguin may release competition for resources among their prey, which may enhance prey growth rates (Chapter 5) and subsequent food supplies for the predator (Chapter 9).

Loripes, and to a lesser extent *Dosinia*, are numerically abundant mollusk species on the intertidal flats of Banc d'Arguin (Honkoop *et al.* 2008; Fig. 6.3). *Loripes* is a predictable food source for its main predator, the red knot, with standing stocks well above the maximum intake rate of 0.1 mg AFDM s^{-1} by red knots on this mildly toxic mollusk in most years (Fig. 8.4C). In contrast, *Dosinia* availability was much more variable between years (Appendix IV, Table IV.1). This variation may be driven by suspended POM availability, which may vary between years and seasons depending on variability in nutrient input by precipitation and Saharan dust transported by trade winds from Africa to America (Lavaud *et al.* 2013, Chapter 2). In years or seasons of relatively high suspended POM availability, recruitment of *Dosinia* may be more successful and subsequent growth may be faster (Fig. 9.4) causing *Dosinia* to be rapidly available in high densities (Fig. 10.3). If *Loripes* would have been absent in *Dosinia*-poor years, far fewer red knots would have been able to make a living at Banc d'Arguin (see Fig. 8.5B).

Despite the fact that *Loripes* availability was relatively constant whereas *Dosinia* was more variable between years, *Loripes* and *Dosinia* availability were negatively correlated (Fig. 8.4C). We suspect that this negative correlation is the result of inter-specific competition for suspended POM (Chapter 5). In years when suspended POM is widely available, the suspension-feeding specialist *Dosinia* is likely to be more efficient in taking advantage of this suspended food than facultative suspension-feeding *Loripes*, especially with regard to the relatively high turn-over rate estimated for *Dosinia* in the previous section. Despite being the inferior competitor for this suspended food, the population of *Loripes* will not go extinct because *Loripes* can also rely on energy produced by endosymbiotic sulphide-oxidizing bacteria. In contrast, when POM conditions are poor, *Dosinia* cannot switch to an alternative diet and will starve to death, whereas *Loripes* can still make a living due to its symbiotic association with sulphide-oxidizing bacteria, while it can also take advantage of the limited POM available (which may even be more than in years with high POM availability due to the lack of competition with *Dosinia* in POM-poor years). This would suggest that the population dynamics in *Dosinia* are largely POM-driven, while population dynamics in *Loripes* are dependent on the abundance of competitively superior *Dosinia*.

Since POM productivity in the clear waters of Banc d'Arguin is likely to be nutrient-limited in most years, which is also reflected by *Loripes* being almost always more abundant than *Dosinia* (Appendix IV, Table IV.1), one might argue that eutrophication may benefit the system because non-toxic organisms like *Dosinia* may then flourish, thus providing better food conditions for secondary consumers. Although this may be true over short time scales, longer periods of high nutrient conditions together with the high light conditions at Banc d'Arguin will favour competitively superior fast-growing algae over seagrasses (Valiela *et al.* 1997), which eventually may lead to an alternative degraded algae-dominated stable state where seagrass cannot return even when nutrient levels are low again (Valentine & Duffy 2006).

Implications for conservation of the Banc d'Arguin seagrass ecosystem

Seagrass meadows rank amongst the ecosystems giving greatest added value in terms of ecological and economic services they provide (Costanza *et al.* 1997). They are highly productive and support a high biodiversity, providing food, shelter and nursery grounds for many animals, including commercially-caught fishery species (Hemminga & Duarte 2000; Larkum, Orth & Duarte 2006; Orth *et al.* 2006). They also act as important carbon and nutrient sinks (Hemminga & Duarte 2000; Duarte, Middelburg & Caraco 2005) and reduce coastal erosion and turbidity by attenuation of waves and currents and by stabilizing the sediment (Larkum, Orth & Duarte 2006). Yet, seagrasses and the services they provide are threatened by the immediate impacts of coastal development and growing human populations as well as by the impacts of climate change and ecological degradation, causing dramatic large-scale losses of these ecosystems worldwide (Orth *et al.* 2006; Waycott *et al.* 2009). This elucidates the necessity of a good understanding of the functioning of seagrass ecosystems for their effective management.

The seagrass beds of Banc d'Arguin not only provide a crucial foraging site for millions of migratory shorebirds using the East Atlantic Flyway (Altenburg *et al.* 1982; Smit & Piersma 1989; Zwarts *et al.* 1998), they also provide a critical habitat for many marine animals, including green turtles (*Chelonia mydas*) and mullets (*Mugil cephalus*), the latter on which indigenous Imraguen fishermen depend for their traditional lifestyle.

In this chapter we show that productivity of macrozoobenthic food is relatively low, which implies that molluscivore migrant shorebirds wintering at Banc d'Arguin are at carrying capacity. This is in contrast with earlier studies by Gils *et al.* (2009) and Kraan *et al.* (2010) who found that populations of molluscivore migrant shorebirds wintering in

Banc d'Arguin are regulated by food conditions encountered at their main staging area, the Wadden Sea. This suggests that a more healthy Wadden Sea, which recently has received the status of World Heritage by UNESCO, may shift the bottleneck in the flyway, the critical phase in the shorebirds' yearly cycle, from the Wadden Sea to Banc d'Arguin. Thus, if the carrying capacity of the Wadden Sea increases, the pressure on authorities of the Parc National de Banc d'Arguin (PNBA) to take adequate measures to protect the carrying capacity of Banc d'Arguin also increases.

Given the narrow margin between macrozoobenthic fauna production and consumption by shorebirds, even the smallest human disturbance during low or high tide could result in a negative energy budget in disturbed shorebirds, leading to population declines. With increasing pressure from the influx of tourists, it will be a great challenge for the authorities of PNBA to minimize tourism-related disturbance within the boundaries of the park, so that tourist can still enjoy the beauty of large flocks of wintering shorebirds foraging on the lush seagrass beds of Banc d'Arguin in the future. However, Banc d'Arguin is also under threat by outside influences, including fisheries, coastal development, and offshore oil exploration and drilling. This elucidates the important role of Mauritanian authorities to also protect the Banc d'Arguin ecosystem services by national regulation that minimizes potential risks caused by human activities outside the boundaries of PNBA.

In this thesis we provide ample evidence that the Banc d'Arguin seagrass ecosystem is regulated bottom-up, through symbiotic relationships between seagrass, lucinid bivalves and their chemoautotrophic endosymbiotic gill-bacteria, and by the availability of suspended POM (Chapter 2, 3, 5), but also top-down by molluscivore shorebirds affecting secondary production rates through release of competition among prey (Chapter 5, 9), which in the case of *Loripes* prey may ultimately enhance seagrass productivity, through reduced sulphide-stress for seagrasses (Chapter 3). The top-down effects exerted by shorebirds again indicate that effective management to keep their high-tide roosts intact and safe from human disturbance is crucial in order to sustain the high carrying capacity of the Banc d'Arguin seagrass beds. Likewise, overfishing may also have top-down trophic cascading effects on the functioning of the seagrass bed community (Valentine & Duffy 2006). For example, overfishing of mullets, important grazers of periphyton at Banc d'Arguin (Michaelis 1993; Wolff *et al.* 1993b), may result in seagrass being overgrown with periphyton, ultimately causing seagrass die off due to light limitation. Unless new studies will show otherwise, we advise the Park authorities to continue their policy to only allow indigenous Imraguen fishermen to harvest fish within the boundaries of PNBA, and to keep excluding fishermen from elsewhere.

In summary, with the new knowledge about the multi-trophic interactions within the seagrass-covered intertidal Banc d'Arguin ecosystem, I hope that this thesis will contribute to a deeper understanding and greater recognition of the importance of seagrass food webs. The seagrass resources are at the basis of the Banc d'Arguin food web and are critical for the management of the valuable natural resources at Banc d'Arguin to be effective.


Carl Zeiss

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Summary
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Summary

Hidden between the East-Atlantic Ocean and the Saharan desert, the Banc d'Arguin comprises a 500 km^2 area of pristine intertidal flats, most of which are covered by dense stands of seagrass (mainly *Zostera noltii*). With over two million wintering shorebirds, this tropical intertidal ecosystem is the most important coastal wintering site along the East-Atlantic Flyway. However, the record feeding densities of shorebirds overwintering here are enigmatic, because the standing stock of macrobenthic invertebrate prey is low. This implies that prey production rates must be high, but at first sight there is no known carbon source for this production, as the clear waters covering the intertidal flats of Banc d'Arguin are nutrient-limited with slow rates of primary production by phytoplankton, while the nutritional value of the large standing stock of seagrass biomass is too low to be of any use for most macrobenthic prey living at Banc d'Arguin.

So the question emerges: what fuels the intertidal seagrass food web of the Banc d'Arguin? In the early 1980s, the discovery of the unique fauna of the deep-sea hydrothermal-vent communities has brought to light an unexpected mode of animal nutrition in which chemoautotrophic bacterial symbionts are maintained within specialized cells of the host animal. In this nutritional association, the bacterial symbionts obtain energy by oxidizing sulphide provided by the animal host, which is used to fix inorganic carbon in to sugars, as opposed to organisms that manufacture their own food through photosynthesis. These sugars fuel both the growth of the symbiotic bacteria and the animal host. This type of symbiosis is now known to also occur in more shallow marine habitats, including intertidal seagrass beds. Within seagrass beds, organic debris is produced at a high rate, which is degraded anaerobically by sulphate-reducing bacteria, causing a build-up of high concentrations of hydrogen sulphide in sediment pore water. Sulphide is toxic to many organisms as it inhibits the functioning of mitochondria and the production of ATP and decreases haemoglobin oxygen affinity. However, by indirectly stimulating sulphide production through high organic matter input and by providing oxygen through radial oxygen release from the roots, seagrass meadows may provide an ideal habitat for benthic invertebrates that live in symbiosis with sulphide-oxidizing endosymbiotic bacteria.

In this thesis we investigate to what extent the high carrying capacity of the intertidal seagrass beds of Banc d'Arguin for migratory shorebirds can be explained by some of their macrobenthic prey making use of sugars provided by symbiotic chemoautotrophic bacteria, a metabolic pathway that was hitherto not fully considered. Carrying capacity is here defined as the maximum population size of a certain species that the environment can sustain for an unlimited period, given the food, habitat, water and other necessities in the environment. We focus on the metabolic pathways used by numerically abundant macrobenthic invertebrates and study their production in tandem with their predation by wintering molluscivore shorebirds. Moreover, we investigate the organizing principles (predation, competition, mutualism) that mould the Banc d'Arguin intertidal seagrass community.

The lucinid bivalve *Loripes lucinalis* (hereafter *Loripes*) dominates the benthic community of the intertidal seagrass beds of Banc d'Arguin, with densities of up to 5,000 individu-

als per m^2. Molecular analysis of DNA extracted from gill tissues of *Loripes* revealed the presence of a dominant bacterial phylotype, related to the sulphide-oxidizing symbionts of other lucinid bivalves, (Chapter 1, box A). Carbon stable isotope analysis showed that on average these chemoautotrophic endosymbionts are responsible for 77% of the carbon diet of *Loripes*, with the remaining part of its diet consisting of benthic microalgae (Chapter 2). Results also revealed that this nutritional contribution varied seasonally, ranging from 84% in early spring (i.e., March) to 69% in autumn (i.e., September). To examine if these changes in diet composition correlate with body condition (size-corrected body mass) and reproductive activity, we investigated seasonal patterns in somatic and gonadal mass investment and gametogenic development in relation to nutrition in *Loripes* (Chapter 2). Size-corrected body and somatic mass varied seasonally and increased from March to October, suggesting food limitation during winter, when the contribution of benthic microalgae to the diet of *Loripes* was relatively high. In contrast, *Loripes* exhibited a semi-annual reproductive cycle characterized by major spawning events in winter between January and February and in summer between July and August. Thus, especially in the winter season the ability to shift to heterotrophic feeding (i.e. benthic microalgae) contributes to growth, reproductive output and survival in *Loripes*, with downstream effects on population dynamics and seagrass community functioning.

Seagrass beds tend to accumulate organic matter, and so it is expected that seagrass beds would build up toxic sulphides, and hence have a limited productivity and diversity. But this is not observed, and the underlying reason for the long-term persistence of seagrass ecosystems is considered an enigma. Using a meta-analysis, a field study, and a laboratory experiment, we tested the hypothesis that a three-stage symbiosis between seagrasses, associated burrowing lucinid bivalves, and their sulphide-oxidizing gill-bacteria releases the sulphide stress for seagrasses (Chapter 3).

The bivalve–sulphide-oxidizer symbiosis indeed reduced sulphide levels and enhanced seagrass production as measured in biomass. In turn, the bivalves and their endosymbionts profited from organic matter accumulation and radial oxygen release from the seagrass roots. These findings elucidate the long-term success of seagrasses in warm waters and offer new prospects for seagrass ecosystem conservation.

To test the hypothesis that in Banc d'Arguin chemosynthetically fixed carbon by *Loripes* and its endosymbionts may fuel the high carrying capacity for migratory shorebirds, we studied the diet of the red knot *Calidris canutus canutus*, the most abundant molluscivore shorebird wintering at Banc d'Arguin. Its habitat of ingesting its hard-shelled prey whole and excreting ingestible shell fragments by defecation, allows a precise methodology that enables the quantitative reconstruction of diet composition through faecal analysis. Analysis of droppings collected in five different years (Chapter 7, 8) showed an average 50% contribution of endosymbiont-bearing *Loripes* to the diet of red knots, thus supporting our hypothesis of a chemosynthesis-based intertidal seagrass food web.

To investigate if production rates of chemosymbiotic *Loripes* and other numerically abundant mollusks are sufficient to sustain the wintering red knot population at Banc d'Arguin we need to know prey growth rates. Mark-recapture experiments are often used to estimate growth rates in bivalves. Yet, traditional marking methods involve the physical handling of the shells and removal from their natural habitat which may lead to disrupted shell growth. This may especially be true for fragile deeply burrowed bivalves like *Loripes*. In Chapter 4, we investigated the suitability of the fluorescent dye calcein as an *in situ* growth marker in burrowing bivalves. Our results showed that *in situ* calcein-marking of *Loripes* with low calcein concentrations (100–200 mg l^{-1}) is a non-invasive and rapid method to determine individual growth rate.

Predators often play key roles in the structuring and organization of ecological communities, with cascading effects down the food web. By alleviating interspecific competition among prey, predators may promote biodiversity and prey growth rates (i.e., productivity), but the precise mechanisms of how predators alter competition have remained elusive. In Chapter 5, we report on a predator-exclosure experiment carried out at the intertidal flats of Banc d'Arguin, providing evidence for a three-level trophic cascade induced by predation by molluscivore red knots that affects pore-water biogeochemistry. Comparing densities of *Loripes* in and outside exclosures we hardly found any difference after a full year of potential predation. This field experiment ran in a year that an alternative bivalve prey, *Dosinia isocardia* (hereafter *Dosinia*), was remarkably abundant. When we compared the relative proportions of *Dosinia* and *Loripes* in the diet with those available in the field, we found that *Dosinia* was much preferred over *Loripes*. We attributed this to *Loripes* being slightly toxic as a result of its sulphur-based metabolism, a hypothesis that is recently confirmed experimentally by feeding trials with captive knots that developed diarrhoea when given a *Loripes*-only diet. In the exclosures, the knots' favourite prey (*Dosinia*) became dominant and reduced the individual growth rate in an alternative prey (*Loripes*). *Dosinia*, a benthic suspension feeder, consumes suspended particulate organic matter (POM), whereas we showed that *Loripes* is a facultative mixotroph, partly living on metabolites produced by sulphur-oxidizing chemoautotrophic bacteria, but also consuming suspended POM (Chapter 2). Reduced sulphide 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 sulphide from the surrounding pore-water. As sulphide is toxic to most organisms, this competition-induced diet shift by *Loripes* may detoxify the environment, which in turn may facilitate other species.

Additional support for top-down regulation of the benthic seagrass community is provided in Chapter 6, where we tested the seasonal depletion hypothesis, which states that benthic biomass at Banc d'Arguin builds up in summer to reach a peak value in September and subsequently is grazed down during the wintering season by shorebirds. Having quantified monthly changes in mollusk biomass over a full year in a seagrass bed, intensively used by wintering red knots, we concluded that molluscivore shorebirds are able to deplete their food stocks in the course of their 'winter'.

§In Chapter 7 we evaluated the potential selective importance of seasonality in pre-reproductive offspring mortality due to predation by wintering red knots on timing of reproduction in *Loripes*. Based on *in situ* determined size- and season-dependent predation and growth rates, we quantified the date-of-birth-dependent offspring predation rate up to size at first reproduction. The reported spawning event in late winter coincides with relatively high offspring predation rates. This suggests that the benefits for *Loripes* to spawn prior to the spring growth season compensate for the relatively high offspring predation rate. However, that spawning ceased during the spring growth season when offspring predation rates were highest, while the second spawning event took place in summer when offspring predation rates dropped to relatively low levels, is consistent with the notion that size- and season-specific predation rates would mould timing of reproduction.

Based on an 8-year time-series on prey abundances, diet choice and predator survival, we showed that red knot annual survival rates were higher in years with higher abundances in their non-toxic prey, like *Dosinia* (Chapter 8). However, as in 6 out of 8 years the non-toxic prey was not abundant enough to satisfy the energy requirements, red knots usually had to fall back on the slightly toxic alternative prey, *Loripes*. Thus, one of the main insights from this thesis is that red knots indeed rely heavily on *Loripes*, but rather differently than we envisioned before. Knots need the mildly toxic prey in years in which non-toxic food is scarce.

In Chapter 9, we report on a field experiment during which we manipulated natural mollusk densities in bare and seagrass habitats in winter and in summer, while measuring growth rates of individually marked bivalves from species that were numerically abundant. Results showed density-dependent growth across both seasons in chemosymbiotic *Loripes* and suspension-feeding *Dosinia* dominating the molluscan community in seagrass sediments, while no density-dependent growth was observed in *Senilia senilis* (hereafter *Senilia*), a suspension-feeding bivalve species dominating the molluscan community in bare sediments. This suggests that the mollusk community of seagrass sediments is close to carrying capacity, whereas that of bare sediments is not, thus supporting the hypothesis that by thinning prey, molluscivore predators at Banc d'Arguin may release competition for resources among their prey, which may enhance prey growth rates and subsequent food supplies for the predator.

In Chapter 10, we quantified production rates and production/average standing biomass ratios (P/B ratios) for *Loripes*, *Dosinia* and *Senilia*. With P/B ratios of 1 yr^{-1} for *Loripes* and 4 yr^{-1} for *Dosinia*, our hypothesis that chemosymbiotic-fuelled bivalves such as *Loripes* would have higher P/B ratios than suspension-feeding bivalves such as *Dosinia* was rejected. Comparison of P/B ratios obtained for *Senilia* with those estimated in the 1980s, showed that these days P/B ratios have increased by a factor 8.5 to a still relatively low P/B ratio of 0.17 yr^{-1}. Together with the observation that standing biomass of suspension-feeding *Senilia* has rapidly increased over the past few decades (Chapter 1,6), we suggest that this increased productivity and standing biomass of *Senilia* may be the result of better suspended particulate organic food conditions at Banc d'Arguin at present.

Finally, we tested whether production rates of *Loripes* and *Dosinia* that dominate the diet of red knots, were sufficient to sustain the red knot population at Banc d'Arguin (Chapter 10). Results revealed that there is a very tight margin between prey production rates and minimal consumption rates necessary for red knots to maintain a balanced energy budget.

Given the observed inter-annual and inter-seasonal variability in prey abundance, this may suggest that in years with relatively poor food conditions, Banc d'Arguin could be the bottleneck in the life cycle of migratory molluscivore shorebirds.

Overall, it can be concluded that chemosynthetic fixation of carbon dioxide by sulphide-oxidizing gill-bacteria of numerically abundant lucinid bivalves, plays a significant role in the carbon flux of the intertidal seagrass food web at Banc d'Arguin. Not only did these chemosymbiotic bivalves make a crucial contribution to the high carrying capacity of this ecosystem for migratory molluscivore shorebirds, they also enhanced seagrass community functioning by releasing sulphide-stress. Conversely, the high predation pressure exerted by the molluscivore migratory shorebirds played an important factor in structuring the Banc d'Arguin seagrass community.

Samenvatting

Verscholen tussen de Oost-Atlantische Oceaan en de Sahara ligt de Banc d'Arguin, een kustgebied bestaande uit 500 km^2 aan ongerepte wadplaten, waarvan de meeste begroeid zijn met een dikke laag zeegras (met name *Zostera noltii*). Dit tropische getijdengebied huisvest meer dan twee miljoen overwinterende wadvogels, waarmee het één van de belangrijkste overwinteringsgebieden is langs de Oost-Atlanstische kust. Hoe deze enorme dichtheden aan fouragerende wadvogels hier kunnen overleven is een raadsel, aangezien de beschikbaarheid aan prooidieren beperkt lijkt. Dit betekent dat de productiesnelheden van deze proodieren hoog moeten zijn. Echter op het eerste gezicht lijkt er geen geschikte koolstofbron beschikbaar die hiervoor zou kunnen zorgen. Het heldere water van de Banc d'Arguin bevat weinig voedingsstoffen: fytoplankton heeft een lage productie, en de voedingswaarde van het alom aanwezige zeegras is voor de meeste bodemdieren te laag.

De vraag is dan ook: wat voedt het met zeegras bedekte waddenecosysteem van de Banc d'Arguin? De ontdekking van de unieke fauna van heetwaterbronnen in de diepzee, begin jaren '80, onthulde een tot dan toe onbekende manier van voedsel vergaren, waarbij chemoautotrofe bacteriële symbionten worden gehuisvest in gespecialiseerde cellen van de gastheer. In deze samenwerking gebruikt de bacteriële symbiont de energie die vrijkomt bij het verbranden van het door de gastheer aangeleverde sulfide om koolstofdioxide om te zetten in suikers, dit in tegenstelling tot fotosynthetiserende organismen die hiervoor licht als energiebron gebruiken. De suikers worden vervolgens voor zowel de groei van de bacterie als van zijn gastheer gebruikt. Een dergelijke symbiose werd later ook ontdekt in ondiepere mariene ecosystemen, waaronder zeegrasvelden. In zeegrasvelden wordt veel organisch afval geproduceerd dat bij gebrek aan zuurstof wordt afgebroken door sulfaat-reducerende bacteriën, wat leidt tot hoge concentraties waterstof-sulfide in het poriewater van het sediment. Sulfide is giftig voor veel organismen doordat het het functioneren van de mitochondria en de productie van ATP remt en doordat het de binding tussen hemoglobine en zuurstof vermindert. Echter, door het indirect stimuleren van sulfide productie door middel van het invangen van veel organisch materiaal in combinatie met de levering van zuurstof vanuit de wortels, kunnen zeegrasvelden een ideaal habitat vormen voor bodemdieren die in symbiose leven met sulfide-oxiderende bacteriën.

In dit proefschrift onderzoeken we in welke mate de hoge draagkracht voor overwinterende wadvogels op de Banc d'Arguin kan worden verklaard doordat sommige in het zeegras levende prooidieren gebruik maken van de suikers die door chemoautotrofe symbiontische bacteriën geleverd worden. We definiëren draagkracht als de maximale populatiegroottte van een bepaalde soort die een gebied kan herbergen voor een ongelimiteerde periode, gegeven het voedsel, habitat, water en andere benodigdheden beschikbaar in dit gebied. We bekijken het metabolisme van de veel voorkomende soorten schelpdieren en bestuderen hun productie en predatie door overwinterende schelpdieretende wadvogels. Verder onderzoeken we de organiserende principes (predatie, competitie, mutualisme) die ten grondslag liggen aan het functioneren van het met zeegras bedekte intergetijdengebied van de Banc d'Arguin.

Het tot de familie van Lucinidae behorende tweekleppige schelpdier *Loripes lucinalis* (hierna *Loripes*) domineert de bodemdiergemeenschap van de met zeegras bedekte wadplaten van de Banc d'Arguin, met dichtheden die oplopen tot wel 5,000 individuen per m^2 (Hoofdstuk 2). Moleculaire analyse van het DNA dat we uit het kieuwweefsel van *Loripes* hebben gehaald, onthulde de aanwezigheid van een dominant bacterieel phylotype, dat familie is van de sulfide-oxiderende symbionten gevonden in andere schelpdiersoorten binnen de familie van de Lucinidae (Hoofdstuk 1, box A). Analyse van stabiele koolstof isotopen liet zien dat deze chemoautotrofe endosymbionten gemiddeld verantwoordelijk zijn voor 77% van het koolstofdieet van *Loripes* en dat de rest van zijn dieet voornamelijk bestaat uit benthische microalgen (voornamelijk eencellige algen die op de zeebodem groeien) (Hoofdstuk 2). De resultaten lieten ook zien dat deze bacteriële contributie varieerde over het jaar, van 84% in het vroege voorjaar (maart) tot 69% in het najaar (september). Om te kijken of deze veranderingen in dieetsamenstelling correleerden met lichaamsconditie (gewicht gecorrigeerd voor grootte) en voortplantingsactiviteit, hebben we gekeken naar eventuele seizoensafhankelijke patronen in het gewicht van somatisch weefsel en van de geslachtsorganen en naar de opbouw van de geslachtsorganen in relatie tot het dieet van *Loripes* (Hoofdstuk 2). Het totale vleesgewicht en dat van alleen het somatische weefsel (gecorrigeerd voor grootte-verschillen) nam toe van maart tot oktober, waarna het weer afnam. Dit suggereert dat voedsel limiterend is in de winter, de periode waarin de contributie van benthische microalgen in het dieet van *Loripes* relatief hoog was. Daarentegen vertoonde *Loripes* een half-jaarlijkse voortplantingscyclus met duidelijke pieken in kuit schieten (het loslaten van de eieren of sperma in de waterkolom) in de winter (januari en februari) en in de zomer (juli en augustus). Het lijkt er dus op dat vooral in de voedselarme winter, de eigenschap om het dieet te kunnen verschuiven naar inname van meer heterotroof voedsel (i.e. benthische microalgen) bijdraagt aan de groei, reproductie en overleving van *Loripes*, wat consequenties heeft voor de populatiedynamica van deze in het zeegras zeer dominante schelpdiersoort en mogelijke gevolgen op het functioneren van het zeegrasecosysteem.

Zeegrasvelden vangen veel organisch materiaal in, waardoor opbouw van giftige sulfides plaatsvindt in de bodem, hetgeen de productiviteit en diversiteit van zeegrasvelden zou moeten beperken, iets dat we echter niet waarnemen in het veld. Het achterliggende mechanisme van hoe deze diversiteit en productiviteit van zeegrasvelden op lange termijn in stand kan worden gehouden, was tot nog toe een raadsel. Met een meta-analyse, een veldstudie en een labexperiment hebben we de hypothese getest dat zeegras, in samenwerking met in het sediment levende, tot de familie van Lucinidae behorende schelpdieren en hun sulfide-oxiderende bacteriën, de sulfide stress voor zeegrasecosystemen opheft (Hoofdstuk 3). De symbiose tussen deze schelpdieren en de sulfide-oxiderende bacteriën leidde inderdaad tot lagere sulfide concentraties en bevorderde zeegrasproductie. In ruil daarvoor profiteerden de schelpdieren en hun kieuw-symbionten van de opbouw van organisch materiaal in de bodem en van zuurstof dat vanuit de wortels van het zeegras de bodem in werd gepompt. Deze bevindingen verklaren het lange termijn succes van zeegrassen in warme wateren en bieden nieuwe mogelijkheden voor de bescherming van zeegrasecosystemen.

Om de hypothese te testen dat chemosynthetisch vastgelegde koolstof (door *Loripes* en zijn endosymbionten) aan de basis ligt van de hoge draagkracht aan wadvogels in de Banc d'Arguin, hebben we het dieet van de kanoet *Calidris canutus canutus*, de meest voorkomende schelpdieretende overwinterende wadvogel in de Banc d'Arguin, bestudeerd. Doordat de kanoet zijn schelpdierprooi in z'n geheel inslikt en de schelpresten weer uitpoept, geven deze poepjes een nauwkeurig beeld van de dieetsamenstelling. Analyse van poepjes verzameld in vijf verschillende jaren (Hoofdstuk 7, 8) liet zien dat gemiddeld 50% van het dieet van kanoeten bestond uit kieuw-symbiont-dragende *Loripes*. Dit bevestigt ons idee van een op chemosynthese gebaseerd Banc d'Arguin zeegrasvoedselweb.

Om te onderzoeken of de productiesnelheden van de chemosymbiotische *Loripes* en andere veel voorkomende schelpdieren genoeg zijn om de overwinterende kanoetenpopulatie te kunnen onderhouden is kennis van de groeisnelheden van deze prooien een vereiste. Merk-terugvang experimenten worden vaak gebruikt om groeisnelheden in schelpdieren te meten. Deze methode vereist echter het fysieke behandelen van de schelpdieren en het verwijderen uit hun natuurlijke habitat wat kan leiden tot verminderde groei, zeker bij fragiele en diep ingegraven schelpdieren zoals *Loripes*. In Hoofdstuk 4 hebben we gekeken of de fluorescerende kleurstof calceine geschikt was als groei 'merker voor ingegraven schelpdieren. Onze resultaten lieten zien dat het *in situ* (in de natuurlijke omgeving) markeren van *Loripes* met lage calceine concentraties (100–200 mg l^{-1}) een geschikte en snelle methode is om individuele groeisnelheden te bepalen.

Predatoren spelen vaak een cruciale rol in de structuur en organisatie van ecologische gemeenschappen wat doorwerkende effecten kan hebben in het voedselweb. Door competitie tussen prooisoorten te verlagen, kunnen predatoren biodiversiteit en groeisnelheden van de prooien (en dus productiviteit) bevorderen. Maar hoe predatoren zulke competitie kunnen beinvloeden is nog veelal onbekend. In Hoofdstuk 5 beschrijven we een predator-exclosure (uitsluiting) experiment dat we hebben uitgevoerd op de wadplaten van de Banc d'Arguin. Dit leverde bewijs leverde voor een trofische cascade over drie niveaus, waarbij als gevolg van predatie door schelpdieretende kanoeten de biochemie van het poriewater veranderde. Na een heel jaar van mogelijke predatie waren de dichtheden van *Loripes* binnen en buiten de exclosures nauwelijks verschillend. Echter, dit veldexperiment was uitgevoerd in een jaar waarin een alternatieve schelpdierprooisoort, *Dosinia isocardia* (hierna *Dosinia*), uitzonderlijk algemeen was. Toen we de relatieve verhouding van *Dosinia* en *Loripes* in het dieet van kanoeten vergeleken met wat er beschikbaar was in het veld, vonden we dat kanoeten een duidelijke voorkeur voor *Dosinia* hadden, ondanks de betere schelp/vlees verhouding van *Loripes*.We veronderstelden dat dit zou kunnen komen omdat *Loripes* wellicht enigszins giftig is door zijn zwavel-metabolisme. Deze hypothese werd recentelijk bevestigd doordat kanoeten in gevangenschap diarree bleken te ontwikkelen als ze alleen *Loripes* te eten kregen. In de exclosures werd de favoriete prooisoort van kanoeten (*Dosinia*) heel dominant, wat tot gevolg had dat de groeisnelheden van de alternieve prooi (*Loripes*) lager werden. *Dosinia* is een zogenaamde"suspension feeder", die gesuspendeerde (rondzwevende) stukjes organisch materiaal (POM) eet, terwijl *Loripes* een facultatieve mixotroof is, die deels leeft van de door symbiontische sulfide-oxiderende bacteriën gesynthetiseerde suikers, maar ook POM eet (Hoofdstuk 2). We vonden verminderde concentraties sulfide in de exlosures, wat verklaard zou kunnen worden doordat de hoge dichtheden aan *Dosinia* binnen de exclosures zorgden voor een sterkere competitie voor POM tussen *Dosinia* en *Loripes*. Deze verhoogde competitie voor POM zou *Loripes* meer afhankelijk kunnen maken van de suikers die geproduceerd wordt door de endosymbiotische bacteriën, wat ertoe leidt dat er meer sulfide wordt opgenomen uit het omliggende poriewater. Als sulfide giftig is voor veel organismen, kan deze door competitie gedreven dieetverandering van *Loripes* de omgeving ontgiftigen, wat weer voordelig kan zijn voor andere soorten.

Nog meer bewijs voor 'top-down' regulatie van de benthische zeegrasgemeenschap wordt geleverd in Hoofdstuk 6, waar we de 'seizoensgebonden depletie' hypothese testten, die stelt dat de biomassa aan bodemdieren in de Banc d'Arguin wordt opgebouwd in de zomer, een piek bereikt in september en vervolgens wordt weggegeten door wadvogels tijdens hun aanwezigheid in de 'winter'. Door de maandelijkse veranderingen in de biomassa van mollusken gedurende een heel jaar te volgen in een zeegrasveld, dat veel door fouragerende kanoeten wordt gebruikt, concludeerden we dat schelpdieretende wadvogels inderdaad in staat zijn om hun voedselbronnen uit te putten gedurende het 'winter' seizoen.

In Hoofdstuk 7 bespreken we de mogelijke selectiekracht van seizoensafhankelijke sterfte onder schelpdieren door predatie door overwinterende kanoeten op de timing van voortplanting in *Loripes*. Op basis van *in situ* bepaalde grootte- en seizoensafhankelijke predatie en groeisnelheden, konden we berekenen wat de geboortedatum-afhankelijke predatiesnelheid was totdat het nageslacht zijn geslachtsrijpe grootte zou bereiken. Schelpjes die werden geboren tijdens de voortplantingspiek in januari en februari (Hoofdstuk 2) ondervonden relatief hoge predatiesnelheden tot het moment dat ze zelf de geslachtsrijpe grootte hadden bereikt. Dit suggereert dat de voordelen voor *Loripes* om zich voort te planten voor het begin van het groeiseizoen (in het voorjaar) het hoge predatierisico voor het nageslacht compenseert. Het kuitschieten werd echter onderbroken in het groeiseizoen, wat ook de periode is waarin de predatiesnelheid het hoogst was, terwijl de tweede voortplantingspiek plaatsvond in de zomer, op het moment dat de predatiesnelheid relatief laag was. Dit is consistent met het idee dat grootte- en seizoensafhankelijke predatiesnelheid invloed kan hebben op de timing van voortplanting.

Op basis van 8 jaar gegevens over voedselaanbod, dieetkeuze en overleving van kanoeten, konden we aantonen dat de overleving hoger was in jaren waarin de niet-giftige prooien, zoals *Dosinia*, meer aanwezig waren (Hoofdstuk 8). In 6 van de 8 jaren was er echter niet genoeg *Dosinia* beschikbaar om aan de energiebehoeftes van kanoeten te voldoen, waardoor deze voor hun dieet deels moesten terug vallen op de enigszins giftige *Loripes*. Eén van de belangrijkste inzichten uit dit proefschrift is daarom dat kanoeten inderdaad sterk afhankelijk zijn van *Loripes*, maar op een andere manier dan we van tevoren hadden gedacht. Kanoeten hebben mild giftige prooien nodig in jaren dat de niet-giftige prooien schaars zijn.

In Hoofdstuk 9 wordt een veldexperiment beschreven waarin we lokaal de natuurlijke dichtheid aan schelpdieren hebben verdubbeld in kale en met zeegras begroeide wadplaten in winter en zomer, waarbij van de algemeen voorkomende schelpdiersoorten de groeisnelheden werd gemeten door middel van een traditionele ‘merk-terugvang’ methode. Dit experiment liet zien dat groei dichtheidsafhankelijk was in zowel zomer als winter voor de chemosymbiotische *Loripes* en de ‘suspension-feeding’ *Dosinia*, de twee dominante schelpdiersoorten in het zeegras habitat, terwijl groei niet dichtheidsafhankelijk was in *Senilia senilis* (hierna *Senilia*), een ‘suspension feeder’ die dominant is in het kale wad. Dit suggereert dat de schelpdiergemeenschap van het zeegras-habitat tegen haar draagkracht aan zit, in tegenstelling tot die van het kale wad. Net als onze bevindingen in Hoofdstuk 5, suggereert dit resultaat dat schelpdieretende vogels middels het uitdunnen van hun prooidieren in het zeegras-habitat, de competitie tussen prooisoorten kunnen verlagen, wat de groeisnelheid van hun prooien en daarmee hun eigen toekomstige voedselaanbod bevordert.

In Hoofdstuk 10 hebben we de productiesnelheden en de verhouding tussen productie en gemiddelde biomassa (de P/B ratio) berekend voor *Loripes*, *Dosinia* en *Senilia*. Met P/B ratios van 1 jr^{-1} voor *Loripes* en 4 jr^{-1} voor *Dosinia*, werd de hypothese verworpen dat chemosynthetisch-gevoede schelpdieren zoals *Loripes* hogere P/B ratios zouden hebben dan ‘suspension-feeding’ schelpdieren zoals *Dosinia*. Een vergelijking van de P/B ratios voor *Senilia* tussen nu en de jaren 80, liet zien dat de P/B ratio 8.5 keer zo hoog is als 30 jaar geleden, maar dat deze nog steeds relatief laag is, met 0.17 yr^{-1}. Naast de hogere P/B ratio is ook de biomassa van *Senilia* aanzienlijk hoger dan 30 jaar geleden (Hoofdstuk 1,6). Dit suggereert dat de toegenomen productiviteit en biomassa van *Senilia* het resultaat zou kunnen zijn van een verhoogde beschikbaarheid van gesuspendeerd organisch materiaal.

Als laatste hebben we onderzocht of de productiesnelheden van *Loripes* en *Dosinia*, de dominante prooisoorten in het dieet van de kanoet, voldoende beschikbaar waren om de populatie van kanoeten op de Banc d’Arguin te kunnen onderhouden (Hoofdstuk 10). Er bleek slechts een minimale marge te zijn tussen de productie van de prooien en de minimale consumptie door kanoeten, die nodig is om een evenwichtig energiebudget te behouden. Gegeven de variatie in prooibeschikbaarheid tussen jaren en seizoenen is de Banc d’Arguin in jaren met relatief slechte voedselomstandigheden mogelijk de ‘flessenhals’ in de levenscyclus van schelpdieretende trekvogels.

Al met al kan geconcludeerd worden dat chemosynthetische fixatie van koolstofdioxide door sulfide-oxiderende bacteriën, die zich in de kieuwen van *Loripes* bevinden, een belangrijke rol speelt in de koolstofstroom van het Banc d’Arguin zeegrasecosysteem. Niet alleen droegen deze chemosymbiotische schelpdieren bij aan de hoge draagkracht van dit ecosysteem voor schelpdieretende trekvogels, ze bevorderden ook het functioneren van de zeegrasgemeenschap door sulfide-stress te verminderen. Andersom speelt de hoge predatiedruk door schelpdieretende trekvogels ook een belangrijke rol in het structureren van het Banc d’Arguin zeegrasecosysteem.

Résumé

Dissimulé entre l'Océan Atlantique Est et le désert du Sahara, le Banc d'Arguin comprend 500 km^2 de vasières intertidales intactes, dont la plupart sont couvertes par des herbiers d'algues denses (notamment *Zostera Noltii*). Avec plus de deux millions de limicoles hivernant, cet écosystème tropical intertidal est le plus important site d'hivernage le long de la voie migratoire Est-Atlantique. Cependant, les concentrations aviaires record observées et la pression trophique associée y sont énigmatiques, car la quantité de biomasse en proies que sont les invertébrés macro-benthiques y est faible. Cela implique que leurs taux de production doivent être élevés, mais il n'y a à première vue pas de source carbonée pour cette production car les eaux claires qui recouvrent les vasières intertidales du Banc d'Arguin sont limitées en nutriments, avec des taux de production de phytoplancton faibles. D'autre part, la valeur nutritionnelle du large stock de biomasse provenant des algues est trop faible pour être d 'aucune utilité pour la plupart des proies macro-benthiques qui vivent sur le Banc d'Arguin.

La question qui se pose est donc la suivante : Qu'est ce qui alimente le réseau trophique des herbiers intertidaux du Banc d'Arguin ? Au début des années 1980, la découverte de la faune singulière des cheminées hydrothermales sous-marines profondes a permis de mettre en évidence un mode de nutrition inattendu par lequel les bactéries chimio-autotrophes symbiotiques se maintiennent dans des cellules spécialisées de l'animal hôte. Dans cette association nutritionnelle, les symbiontes bactériens obtiennent leur énergie en oxydant des sulfures provenant de l'hôte, ce qui est utilisé pour fixer le carbone inorganique en sucre, contrairement aux organismes qui produisent leur propre nourriture par la photosynthèse. Ces sucres stimulent à la fois la croissance des bactéries symbiotiques et celle de l'animal hôte. Ce type de symbiose est également connu dans les environnements marins moins profonds, y compris dans les herbiers intertidaux. Dans les herbiers, les débris organiques qui sont produits en masse sont dégradés en conditions anaérobies par les bactéries sulfato-réductrices, ce qui engendre une accumulation de sulfures d'hydrogène dans les eaux porales des sédiments. Les sulfures sont toxiques pour de nombreux organismes car ils inhibent le fonctionnement des mitochondries et la production de l'ATP, et ils diminuent l'affinité de l'hémoglobine à l'oxygène. Cependant, en stimulant indirectement la production de sulfure par le fort apport en matière organique, et en procurant de l'oxygène par le relargage radial d'oxygène par les racines, les herbiers sont susceptibles de fournir un habitat idéal pour les invertébrés benthiques qui vivent en symbiose avec des bactéries endosymbiotiques oxydant les sulfures.

Dans la présente thèse, nous avons cherché a déterminer si la capacité portante des herbiers intertidaux du Banc d'Arguin pour les limicoles migrateurs peut être expliquée par le fait qu'une partie des proies macro-benthiques utilise les sucres fournis par les bactéries chimio-autotrophes symbiotiques, une voie métabolique jusqu'alors inexplorée. La capacité portante est ici définie comme la taille maximum de la population d'une espèce donnée, que l'environnement peut supporter pour une période de temps illimitée, sur la base de la nourriture, de l'habitat, de l'eau et d'autres nécessités environnementales. Notre étude est focalisée sur les voies métaboliques utilisées par le plus grand nombre d'invertébrés macrobenthiques et nous avons étudié leur production en tandem avec leur prédation par les limicoles hivernants. Par ailleurs, nous avons examiné les principes d'organisation (prédation, compétition, mutualisme) qui régissent la communauté des herbiers intertidaux du Banc d'Arguin.

Le bivalve de la famille Lucinidae *Loripes lucinalis* (ci-après *Loripes*) domine la communauté benthique des herbiers intertidaux du Banc d'Arguin, avec des densités allant jusqu'à 5,000 individus au m^2 (Chapitre 2). L'analyse moléculaire de l'ADN extraite des branchies de *Loripes* révèle la dominance de phylotypes bactériens liés aux symbiontes oxydants les sulfures d'autres bivalves Lucinidae (Chapitre 1, Encadre A). Les isotopes stables du carbone montrent qu'en moyenne, ces endosymbiontes chimio-autotrophes sont responsables pour 77% de la diète carbonée de *Loripes*, la part restante consistant en des micro-algues benthiques (Chapitre 2). Les résultats montrent également que cette contribution nutritionnelle varie saisonnièrement, allant de 84% au début du printemps (i.e., Mars) à 79% en automne (i.e., Septembre). Afin de déterminer si ces changements de composition de l'alimentation sont corrélés à la condition physique (la masse corporelle corrigée de la taille) et à l'activité reproductrice, nous avons examiné les distributions saisonnières en investissement de la masse par les gonades et les tissus somatiques, et en développement gamétogénique en fonction de la nutrition chez *Loripes* (Chapitre 2). La masse corporelle corrigée de la taille et la masse gonadique varient saisonnièrement et augmentent de Mars à Octobre, ce qui suggère une limitation en nourriture durant l'hiver, lorsque les contributions en micro-algues benthiques dans l'alimentation de *Loripes* sont relativement élevées. Au contraire, *Loripes* a démontré un cycle reproductif semi-annuel caractérisé par des évènements de ponte marquants pendant l'hiver entre Janvier et Février et en été entre Juillet et Aout. Ainsi, la capacité de *Loripes* à passer à un mode de nutrition hétérotrophe (les micro-algues benthiques) pendant l'hiver contribue à la croissance, à l'activité reproductrice et à la survie de l'espèce, avec des effets subséquents sur la dynamique de la population et le fonctionnement de la communauté d'algues.

Les herbiers ont tendance à accumuler la matière organique, et il est donc attendu qu'ils aient tendance à accumuler les sulfures toxiques, limitant ainsi la productivité et la diversité. Mais ceci n'est pas observé, et la raison sous-tendant la pérennité des écosystèmes des herbiers reste une énigme. En utilisant une méta-analyse, une étude de terrain et une expérience en laboratoire, nous avons testé l'hypothèse qu'une symbiose en trois stades entre les algues, les bivalves fouisseurs Lucinidae et leurs bactéries branchiales oxydant les sulfures permette de réduire le stress sulfurique pour les algues (Chapitre 3). La symbiose bivalve-oxydant des sulfures a effectivement produit une réduction de la quantité de sulfures réduits et une augmentation de la production en biomasse d'algues. En outre, les bivalves et leurs endosymbiontes ont profité de l'accumulation de matière organique et du relargage radial d'oxygène par les racines des algues. Ces résultats ont permis de comprendre le succès à long-terme des herbiers en eaux chaudes et ouvrent de nouvelles pistes pour la conservation des écosystèmes des herbiers marins.

Pour tester l'hypothèse que le carbone fixé par *Loripes* et ses endosymbiontes alimente la forte capacité portante pour les limicoles migrateurs, nous avons étudié la diète du bécasseau maubèche *Calidris canutus canutus*, qui est le limicole molluscivore hivernant le plus abondant sur le Banc d'Arguin. Son habitude d'ingérer complètement la coquille de ses proies et d'excréter les parties indigestes par défécation permet de mettre en place une méthodologie précise dont le but est de reconstituer quantitativement la composition de la diète par l'analyse fécale. L'analyse des fientes collectées pendant 5 ans (Chapitre 7, 8) a démontré une contribution moyenne de 50% de *Loripes* à l'alimentation des bécasseaux maubèches, ce qui supporte notre hypothèse du réseau trophique des herbiers basé sur la chimiosynthèse.

Pour examiner si les taux de production de *Loripes* et d'autres mollusques présents en grand nombre sont suffisants pour soutenir l'hivernation de la population de bécasseaux maubèches au Banc d'Arguin, il est nécessaire de connaitre les taux de croissance de ses proies. Les expériences de marquage-recapture sont souvent utilisées pour estimer les taux de croissance des bivalves. Cependant, les méthodes traditionnelles de marquage nécessitent de manipuler les bivalves et ainsi de les retirer de leur habitat naturel, ce qui peut entrainer une perturbation de la croissance des coquilles. Ceci est particulièrement tangible pour les fragiles *Loripes* qui sont enfouis profondément. Dans le Chapitre 4, nous avons examiné la pertinence de la calcéine fluorescente comme marqueur de croissance *in situ* des bivalves fouisseurs. Nos résultats montrent que le marquage de *Loripes* en utilisant des faibles concentrations de calcéine est une méthode non-invasive et rapide pour déterminer les taux de croissance individuels.

Les prédateurs jouent souvent un rôle clé dans l'organisation et la structuration des communautés écologiques, avec des effets se répercutant dans la chaine trophique. En diminuant la compétition interspécifique entre proies, les prédateurs peuvent stimuler la biodiversité et la croissance des proies (i.e., la productivité), mais les mécanismes précis qui régissent comment les prédateurs modifient la compétition reste élusifs. Dans le Chapitre 5, nous présentons les résultats d'une expérience d'exclusion de prédateur effectuée sur les vasières du Banc d'Arguin, qui a permis de démontrer l'existence d'une cascade trophique en trois stades induite par la prédation des bécasseaux maubèches en modifiant la biogéochimie des eaux porales. Les densités de *Loripes* dans et hors des zones d'exclusions ont été trouvées non significativement différentes après une année entière de prédation potentielle. Cette étude de terrain a été menée une année où une proie alternative, le bivalve *Dosinia isocardia* (ci-après *Dosinia*), était remarquablement abondante. En comparant les proportions relatives de *Dosinia* et *Loripes* dans la diète avec ceux disponibles dans l'environnement, nous avons constaté que *Dosinia* était préférée à *Loripes*. Nous attribuons cette observation au fait que *Loripes* est faiblement toxique du à son métabolisme lié aux sulfures, une hypothèse qui s'est trouvée récemment confirmée par des essais d'alimentation de bécasseaux captifs, qui ont développé une diarrhée avec une diète uniquement constituée de *Loripes*. Dans les zones d'exclusion, la proie favorite des bécasseaux (*Dosinia*) est devenue dominante et a réduit le taux de croissance individuel de *Loripes*. *Dosinia*, qui se nourrit de suspension benthique, consomme de la matière organique particulaire (MOP) en suspension, tandis que nous avons montré que *Loripes* est un mixotrophe facultatif, qui se nourrit de métabolites produits par des bactéries chimio-autotrophes oxydant les sulfures, mais également de MOP en suspension (Chapitre 2). Les plus faibles concentrations en sulfures dans les zones d'exclusion suggèrent que sans la prédation des *Dosinia*, une plus forte compétition pour la MOP en suspension force *Loripes* à utiliser l'énergie produite par les bactéries endosymbiotiques, ce qui entraine l'utilisation des sulfures des eaux porales avoisinantes. Comme les sulfures sont toxiques pour la plupart des organismes, cette modification de la diète induite par la compétition peut contribuer à la détoxification de l'environnement et ainsi favoriser d'autres espèces.

Un autre argument en faveur de la régulation en amont des communautés benthiques des herbiers est apporté dans le Chapitre 6, ou nous avons testé l'hypothèse de la déplétion saisonnière, qui dicte que la biomasse benthique sur le Banc d'Arguin se constitue en été et est maximale en septembre, pour être ensuite consommée pendant la saison d'hivernage par les limicoles. Nous avons quantifié les changements mensuels de la biomasse des mollusques sur une année dans un herbier utilisé intensément par les bécasseaux hivernants et nous avons conclu que les limicoles sont aptes à diminuer leur stock de nourriture pendant l'hivernage.

Dans le Chapitre 7, nous avons évalué l'importance sélective potentielle de la saisonnalité dans la mortalité juvénile pré-reproductive due à la prédation par les bécasseaux hivernants sur le timing de la reproduction de *Loripes*. En se basant sur des déterminations *in situ* de la prédation et des taux de croissance en fonction de la taille et de la saison, nous avons quantifié le taux de prédation des juvéniles en fonction des dates de naissance jusqu'à la taille de la première reproduction. Les évènements de ponte observés à la fin de l'hiver coïncident avec des taux de prédation de juvéniles relativement élevés. Ceci suggère que les bénéfices pour *Loripes* de pondre avant la saison de croissance printanière compensent le taux relativement élevé de prédation. Cependant, le fait que la ponte cesse lors de la saison de croissance printanière alors que les taux de prédation juvénile sont les plus forts, tandis que le second évènement de ponte a lieu en été lorsque les taux de prédation juvénile sont relativement bas concorde avec le fait que les taux de prédation spécifiques à la taille et à la saison influenceraient le timing de la reproduction.

En se basant sur une série temporelle de 8 ans des abondances des proies, des choix alimentaires et de la survie des prédateurs, nous avons montré que les taux de survie annuels des bécasseaux maubèches sont plus élevés les années de plus forte abondance en proies non-toxiques, comme *Dosinia* (Chapitre 8). Cependant, pour 6 des 8 années, les proies non-toxiques n'étant pas assez abondantes pour satisfaire leurs besoins énergétiques, les bécasseaux maubèches ont du se rabattre sur leur proie plus légèrement toxique, *Loripes*. Ainsi, un des résultats majeurs de cette thèse est que les bécasseaux maubèches dépendent en effet largement de *Loripes*, mais de manière plutôt différente de ce qu'on avait précédemment envisagé. Les bécasseaux ont besoin des proies légèrement toxiques les années où les proies non-toxiques sont rares.

Dans le Chapitre 9, nous présentons les résultats d'une campagne de terrain durant laquelle nous avons manipulé les densités naturelles des mollusques dans des habitats avec et sans algues en été et en hiver, tout en mesurant les taux de croissance de bivalves marqués individuellement parmi les espèces les plus abondantes. Les résultats montrent une croissance en fonction de la densité de *Loripes* et *Dosinia* pour les deux saisons, espèces qui dominent la population de mollusques dans les herbiers, alors qu'aucune croissance en fonction de la densité n'a été observée chez *Senilia senilis* (ci-après *Senilia*), bivalve se nourrissant de suspension qui domine la population de mollusques des sédiments sans algues. Cela suggère que la communauté des mollusques des sédiments à herbiers est proche de la capacité portante, tandis que celle des sédiments sans algues ne l'est pas, ce qui corrobore l'idée qu'en diminuant le nombre de proie, les prédateurs molluscivores du Banc d'Arguin puissent stimuler la compétition entre proies pour les ressources et ainsi augmenter leur taux de croissance et l'apport de nourriture pour le prédateur.

Dans le Chapitre 10, nous avons quantifié les taux de production et le rapport production/biomasse (rapport P/B) pour *Loripes*, *Dosinia* et *Senilia*. Avec des rapports P/B de 1 yr^{-1} pour *Loripes* et 4 yr^{-1} pour *Dosinia*, notre hypothèse que des bivalves chimio-symbiotiques comme *Loripes* auraient des rapports P/B supérieurs à des bivalves se nourrissant de suspension comme *Dosinia* s'est trouvée rejetée. La comparaison des rapports P/B de *Senilia* avec ceux estimés dans les années 1980 a montré que les rapports P/B récents ont augmente d'un facteur 8.5 avec un rapport P/B relativement faible de 0.17 yr^{-1}. Avec l'observation que la biomasse de *Senilia* a rapidement augmentée au cours des dernières décennies (Chapitres 1, 6), nous suggérons que cette augmentation de productivité et de biomasse de *Senilia* puisse être le résultat de meilleures conditions de MOP en suspension sur le Banc d'Arguin.

Finalement, nous avons testé si les taux de production de *Loripes* et *Dosinia* qui dominent l'alimentation des bécasseaux maubèches étaient suffisants pour maintenir la population de bécasseaux du Banc d'Arguin. Les résultats ont révélé qu'il a une marge très tenue entre le taux de production des proies et les taux de consommation minimaux nécessaires pour maintenir la balance énergétique des bécasseaux. D'après la variabilité interannuelle et inter-saisonnière en abondance des proies observée, cela tend à suggérer que lors des années de pauvres conditions alimentaires, le Banc d'Arguin puisse être le goulot d'étranglement du cycle de vie des limicoles migrateurs.

D'un point de vue général, nous pouvons conclure que la fixation par la chimiosynthèse du dioxyde de carbone par les bactéries branchiales oxydant les sulfures des abondants bivalves Lucinidae joue un rôle significatif dans les flux de carbone de la chaine trophique des herbiers intertidaux du Banc d'Arguin. Non seulement ces bivalves chimio-symbiotiques apportent une contribution cruciale à la capacité portante de cet écosystème pour les limicoles migrateurs, mais ils améliorent également le fonctionnement de la communauté des herbiers en diminuant le stress sulfurique. A contrario, la forte prédation exercée par les limicoles migrateurs est un facteur important de la structuration des herbiers marins du Banc d'Arguin.

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A nice way to escape from the stress of work is doing sports. For many years I have joined the NIOZ-football team and I want to thank all players for putting up with me when getting over-excited during the game. Richard, and previously Cees, thanks for being such great Captains. Peter, thanks for coaching us during most years, the start/end-of- the-football-season parties at your place were memorable. Hanzie Polderman, bedankt voor de moie avonden in 'Ons Genoegen', ik denk dat de derde-helft van het NIOZ-team jouw baromzet geen windeieren heeft gelegd! Sheep-man, stand-in goalie and current coach Paul Hoogendijk, niet alleen ben je een fantastiche vent, ook ben je de enige Texelaar die altijd vond dat we beter waren dan de tegenstander, zelfs als we met 10-0 verloren! Bedankt voor je vertrouwen in ons en voor je gezelligheid. Apart from the NIOZ-football team I also joined the NIOZ-volleyball-team. Jan-Berend, Mardik, Bruno, Jenny, Allert, Tamar, Roos, Richard, it was a pleasure to play with you all! Gerhard, Biem, Piet, Berry, Edi, Allert: thanks for the nice games of tennis we played over the years!

I also would like to thank the MEE-group for the fantastic working atmosphere! Sjoerd, Roeland, Jim, Allert, Thomas, Anita, Anne, Eelke, Jeltje, Anouk, David, Tanya, Jenny, Andreas, Jaap, Tamar, Roos, Sofia, Lisa, Anita, Piet, Lodewijk, Maarten, Bernard, Vania, Stefania, Cees, Jorge, Jutta, Pieternella, Geert, Casper, Tineke, Henrike, Joanna, Sylvia, Jeremy, Theunis, Marc, Henk, Jan (van Gils), Jan (Drent), Gerard (Nieuwland), Rolf, Gerard (Duineveld), Rob (Witbaard), Rob (Dekker), Petra, Pieter, Eldar, Sander, Job, thanks for all your input, presentations, discussions, and shared coffee, lunch and tea-breaks. Casper, Piet, Eldar, Roos, Tamar, thanks for being great room-mates!

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Hein, bedankt dat je me de kans hebt geboden om de cursus 'Marine Biology and Oceanography 2013' te helpen organiseren. Niet alleen was het geld van harte welkom, ook was het een leerzame en vooral leuke ervaring!

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Rik, thanks for driving us up and down to Hamburg with a little D-tour to lovely Bourthange. I share your passion for good coffee and hope that you won't take 'the' coffee-machine with you to Berlin. Cecile, my lovely French connection! Thanks for translating my thesis-summary into French. I cannot believe you even enjoyed doing it! If you ever decide to change jobs I suggest you to become a translator. Given your speed you can make good money! Rik, Cecile, I wish you and your kids a great future in Berlin, and given its Berlin and not Den Helder, I promise I will drop by someday.

Marije, bedankt voor het maken van de prachtige illustratie op de cover van dit proefschrift. Je bent een professional! Jesse, als er iemand altijd klaar stond om mij te helpen dan was jij het wel. Jouw gastvrijheid is ongeëvenaard. Ik heb genoten van je kookkunsten en van de gezellige avonden bij jullie in de tuin samen met de kinderen en van de lekkere biertjes die je voor me hebt getapt in de 12 Balcken. En wat was het fijn dat Veerle het afgelopen jaar regelmatig bij jullie thuis kon komen spelen met Raven, zodat ik nog wat uurtjes kon pakken om aan dit proefschrift te werken. Ik koester onze muzikale roes bij het concert van de Tindersticks in de duinen van Vlieland. Zonder jou en Marije was mijn leven op Texel een stuk minder cultureel en smaakvol geweest.

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Craig and Julie, thanks for inviting us to the most romantic wedding ever. You two really make a fantastic couple. I wish you and Lauren all the best in Groningen, and I look forward to visit you there.

Craig, you're the funniest character I have ever met. Going for a beer with you always ends up in the worst scenario, but I loved every minute of it. Your hospitality is exceptional and so are the lovely curries that you have made for me over the years. However, given your blocked sink I do have to advise you to cut up the vegetables a bit smaller. Watching a game of Spurs at White Hart Lane with you and your dad was an unforgettable experience. I still don't know how you managed to drink so much without pissing in your pants when

Lennon scored the winning goal in extra time, but I guess it has to do with your Scottish bloodline. It's been an honour to be your paranimf, and I am very pleased that you and Allert will be my paranimfs. Craig, thank you for being such a loyal friend!

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Den Burg, October 2013









BLUES BROTHER CASTRO


الحظيرة الوطنية لحوض آركين
PARC NATIONAL DU BANC D'ARGUIN
مركز اويك
Poste d'IWIK
الاتصال عن طريق اللاسلكى متوفر للمزيد
من المعلومات اتصلوا بحارس الحظيرة خلال
عبوركم آزفال، اتبعوا خط السير المعهود يمنع اخذ الاغراض الاثرية
Vacations radios possibles (se renseigner auprès du garde du Parc)
Ne pas s'écarter des traces pour traverser l'Azefall
Collecte d'objets préhistoriques interdite





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List of publications

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van der Geest, M., van der Lely, J.A.C., van Gils, J.A. & Piersma, T. (submitted) Density-dependent growth in three tropical intertidal bivalves: importance of feeding style, habitat and season.

van der Geest, M., Lok, T., van Gils, J.A. & Piersma, T. (submitted) Size- and season-specific predation moulds timing of reproduction in a tropical marine bivalve.

van der Geest, M., Sall, A.A., Ely, S.O., Nauta, R.W., van Gils, J.A. & Piersma, T. (in revision) Nutritional and reproductive strategies in a chemsoymbiotic bivalve living in a tropical intertidal seagrass bed. *Marine Ecology Progress Series*.

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