The tell-tale isotopes

Towards indicators of the health of the Wadden Sea

Jeltje Jouta

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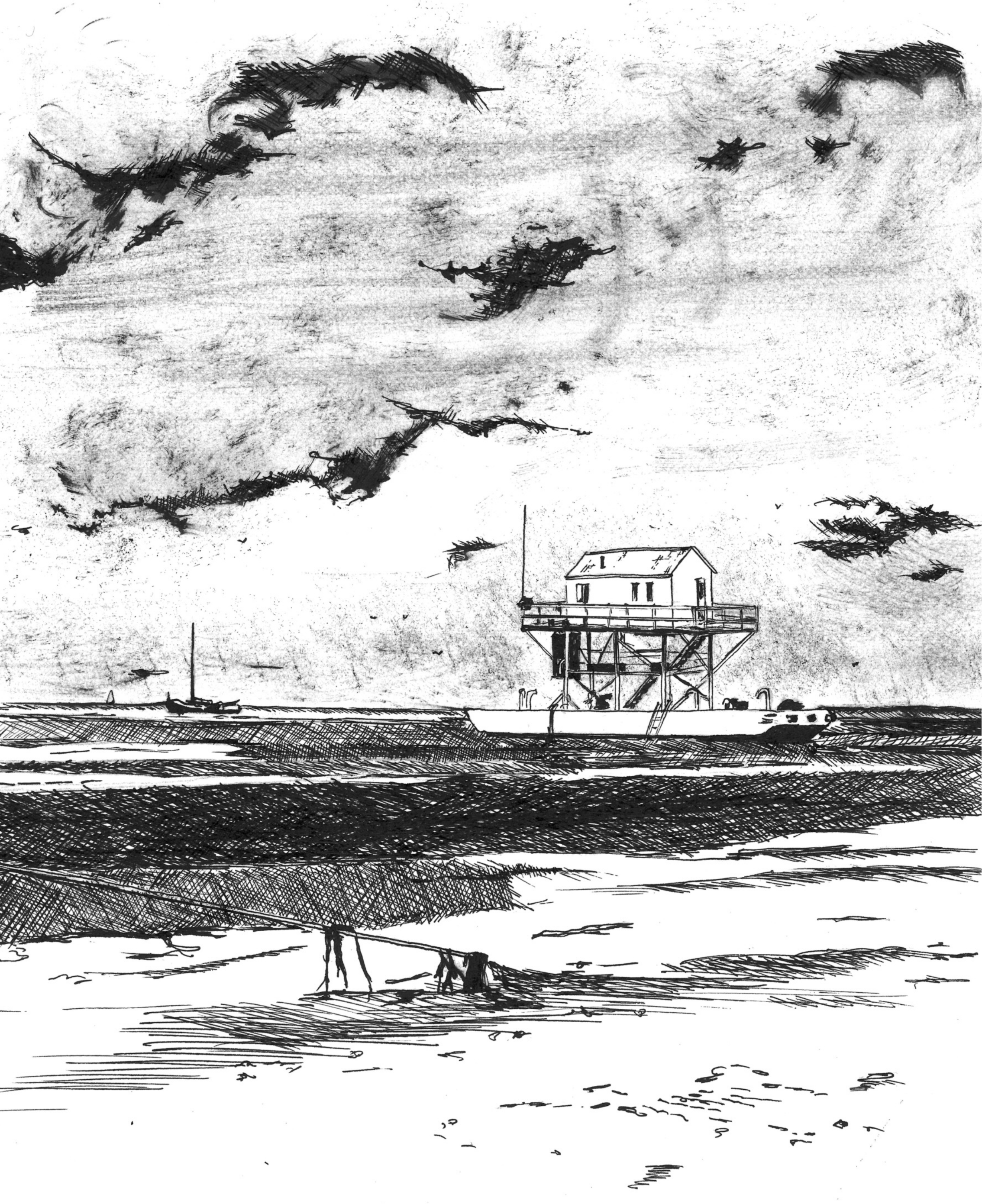

CHAPTER 1

General introduction

Jeltje Jouta

With awe I look at the Wadden Sea. So stirred, so much pressure, so impressionable, yet tough. Perhaps – currently – in another state than what it has been before, but still impressive. With growing amazement I have come to realize how good she[1] manages to stay afloat, how flexible she must be. "*But why does his heart not stop beating?! Why does it not stop!?*" These are the last two sentences of a story by Edgar Allen Poe, the story touched me and I therefore refer to it in my thesis title. In this thesis I search for indicators that represent the state of health of the Wadden Sea ecosystem.

Pardon me! Ecosystem health?

An ecosystem is a complex set of relationships formed by the interactions of living sources and their physical environment. Ecosystems are the worlds' ultimate life-support and ecosystem health is crucial for the success and survival of humans (Karr 1996, McCann 2000), which is likely the cause of the urge to understand and protect ecosystems and explains the wish to keep an ecosystem in a strong, resilient, pristine and healthy state[2].

Ecosystems have a changeable nature and a stable ecosystem is not static, but faces changes that can often be defied by the ecosystem (McCann 2000). Ecosystems assemble over time, developing via succession from a simple and young pioneer stage towards a complex and old climax stage. During succession natural, more or less predictable and orderly changes occur in the species composition and food web structure of a community over time (Odum 1969, Pandolfi, Sven Erik & Brian 2008). Note that the standards of ecosystem health are different when comparing a pioneer and climax ecosystem. An ecosystem in a pioneer stage is thus not (necessarily) in a worse ecosystem health compared to a climax stage. Natural dynamics are a not a reason either to

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[1] For me the Wadden Sea is feminine (and most French people will likely agree), but note that the old man of Edgar Allan Poe was masculine.

[2] The term 'ecosystem health' is criticized for unjustly comparing to human health (e.g. Suter 1993), yet I will use this term intuitively. As you might have noted, I feel like comparing the state of ecosystems with the state of human beings that can both be described as young, old, erratic, stable, poor, rich, healthy or unhealthy. Being healthy contributes to the chance to survive. Just as there is a desire to keep all your loved ones healthy, we also want to keep an ecosystem healthy and alive (possibly imprimis for the survival of mankind). Differences between ecosystem health and human health are profound, for instance, humans have clear boundaries such as a skin while ecosystems clearly have not. The main argument against using the term ecosystem health is because of the absence of a fixed index. Despite the existence of many ecosystem measures, no standard set of ecosystem measures are defined to indicate ecosystem health.
Note that ecological health and ecosystem health are not the same. The term ecological health is used in medicine to refer to exposure of chemicals in an environment, like pesticides or smoke. Whereas ecosystem health concerns the structural and functional condition of biological systems (Karr 1996, Rapport, Costanza & McMichael 1998).

assign a state of an ecosystem the label 'unhealthy'. However, human exploitation of nature can impact an ecosystem so violently that, as a consequence, the ecosystem faces degradation. The ecosystem can react in three ways; the degradation can be (a) temporary because the ecosystem shows resilience or (b) temporary since it is followed up by restoration of the ecosystem with help of human efforts, or (c) permanent. In the second and third case, degradation results in an ecosystem with a lower ecosystem health. But how do we qualify ecosystem health?

Numerous measures for ecosystem health are developed and among them are named; biodiversity, net primary production, species richness, landscape mosaics, flow of energy, number of links in a food web and shape of food webs (Xu, Tao, Dawson et al. 2001, Hooper, Chapin, Ewel et al. 2005, Boero & Bonsdorff 2007, Costanza, Fisher, Mulder et al. 2007, Mancinelli & Vizzini 2015, Bird, Perissinotto, Miranda et al. 2016). These ecosystem measures can be translated or merged into bigger qualitative terms such as the comprehensive definitions of the 'state of an ecosystem', 'ecosystem functioning' or 'ecosystem health' (Hobbs & Norton 1996, Slocombe 1998). Despite the existence of many ecosystem measures, no standard set of ecosystem measures are yet defined to indicate ecosystem health. In this thesis I attempt to use food web structure as an ecological measure for ecosystem health.

Food webs

Food is one of the most important factors in life, and that is how Charles Elton (1927) invented food webs and appointed them as a valuable way to describe and compare ecosystems. The analysis of food web structures has become a commonly accepted method to determine the state of ecosystems and provide complex yet manageable representations of biodiversity, species interactions, ecosystem structure and ecosystem function (Dunne, Williams & Martinez 2002). When assessed over time or space, food web structures can instruct us about the effects of biodiversity loss, including secondary and cascading extinctions, and show the sensitivity of species and their connectance (Dunne et al. 2002).

A food web is a diagram depicting who eats whom in ecosystems. Figure 1.1 depicts a conceptual food web, with primary producers such as plants or algae positioned at the bottom of the food web, which are eaten by primary consumers (herbivores) such as beetles, geese or mud snails, which are eaten by mesopredators like passerine birds or fish of intermediate size and finally at the top of the food web are the toppredators such as raptors and dolphins. Food webs can be ordered in trophic levels, shown in table 1.1, with the trophic level of an organism being determined by the number of 'who-eat-who' feeding links that have passed.

Note that, although the detritivorous pathway plays a critical role in organizing and sustaining ecosystem (Odum 1969, Moore, Berlow, Coleman et al. 2004), I simplified figure 1 by simply drawing arrows from food web elements to 'detritivore 1', hereby skipping the step of 'dead organic matter'. Although the detritivorous pathway recycles energy of dead organic matter, most energy is transformed into heat and lost in the system (Krebs 2001). With increased trophic level, the flow of energy therefore typically becomes smaller, resulting in a pyramid shape, called a food pyramid.

Most ecosystems are regulated by top-down and bottom-up control or by the interaction of the two, meaning that the ecosystem is respectively regulated by predation control and by nutrients and primary productivity, or by both (Menge 2000, Eriksson, van der Heide, van de Koppel et al. 2010). In marine systems, the joint effect of fisheries and eutrophication should be considered very seriously in marine nature management (Eriksson et al. 2010).

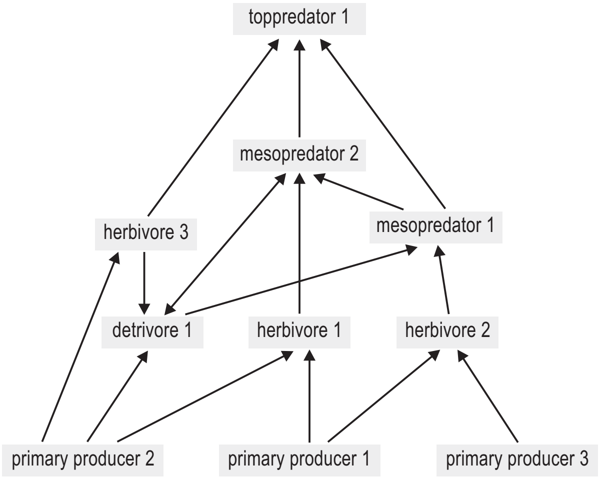


Figure 1.1: Conceptual food web in a pyramid form.

Table 1.1: Organisation of food webs.

First trophic level	Primary producers		'Plants & Algae'
Second trophic level	Primary consumers		Herbivores
Third trophic level	Secondary consumers	Mesopredator	Carnivores[1]
Fourth trophic level	Tertiary consumers	Toppredator[2]	Higher carnivores[3]

1: Insects parasitoids can also be part of this group.
2: Although most food webs are limited to four trophic levels, fifth or more trophic levels may occur, with the position of toppredators logically shifting to the highest level.
3: Insect hyperparasites can also be part if this group. (Krebs 2001)

Food webs can intertwine and can either have a more or less closed or an open nutrient cycle. Whereas in closed systems herbivores and detritivores depend on locally regulated nutrient cycling, in open systems external nutrient input are a major influence driving of the food web. Moreover mobile species, such as migratory birds, can shift between ecosystems, hereby linking several food webs. Although the shape of food webs is typically pyramid-shaped, external input nutrient input and ecosystem transcending processes might result in other food web shapes, with for instance more emphasis in the top part of the food web. Reconstruction of food webs, with help of stable isotopes analysis, can be used as a means to study the state of an ecosystem (Augusto, Tassoni, Ferreira et al. 2015, Mancinelli et al. 2015, Bird et al. 2016).

Before I examine ways to assay the structure of food webs using stable isotopes, we need to share the caveat that food relationships are an important, but not the only one, relationship making up an ecosystem. There are additional non-trophic ecological interactions such as parasitism, competition, mutualism and ecosystem engineering which also strongly feed back on ecosystems and even food web structure (Olff, Alonso, Berg et al. 2009, van der Zee, van der Heide, Donadi et al. 2012).

Stable isotopes

Stable isotopes of nitrogen and carbon are powerful and frequently used tools to reconstruct food webs (Peterson, Howarth & Garritt 1985, Post 2002). Stable isotopes are used to determine the food web position of species or species groups, making it possible to create a two-dimensional food web (Figure 1.2). In food web ecology, food web reconstructions based on stable isotopes mainly yields to qualitative food webs.
The stable nitrogen isotope ^{15}N is used to elucidate the trophic structure, displayed on the vertical axis in a food web, while the stable carbon isotope ^{13}C provides information on the input of carbon sources at the base of the food web and can thus infer the energy transfer through food webs, displayed on the horizontal axis in a food web (pyramid) (Figure 1.2) (Peterson & Fry 1987, Post 2002, Middelburg 2014).

The trophic position of consumers can be estimated by level of the two isotopes relative to a fixed standard, hence we talk about $\delta^{15}N$ (delta ^{15}N) and $\delta^{13}C$. The level of the $\delta^{15}N$ value of consumers relative to the $\delta^{15}N$ value of the primary sources of the food web, often show an 'enrichment' by ~3.4‰ in $\delta^{15}N$ per trophic level. ^{13}C does not show 'enrichment'; instead the $\delta^{13}C$ of plants varies because of different $\delta^{13}C$ values in inorganic carbon substrate (atmospheric carbon dioxide or dissolved inorganic carbon) and because of the involvement of different carbon isotope fractionations during C3 or C4 photosynthesis pathways. Thus, in contrast to nitrogen, the ratio of carbon isotope ($\delta^{13}C$) does not change substantially as carbon moves through the food web, making $\delta^{13}C$ ideal to determine the ultimate sources of consumers (Peterson et al. 1987, Post 2002, Middelburg 2014).

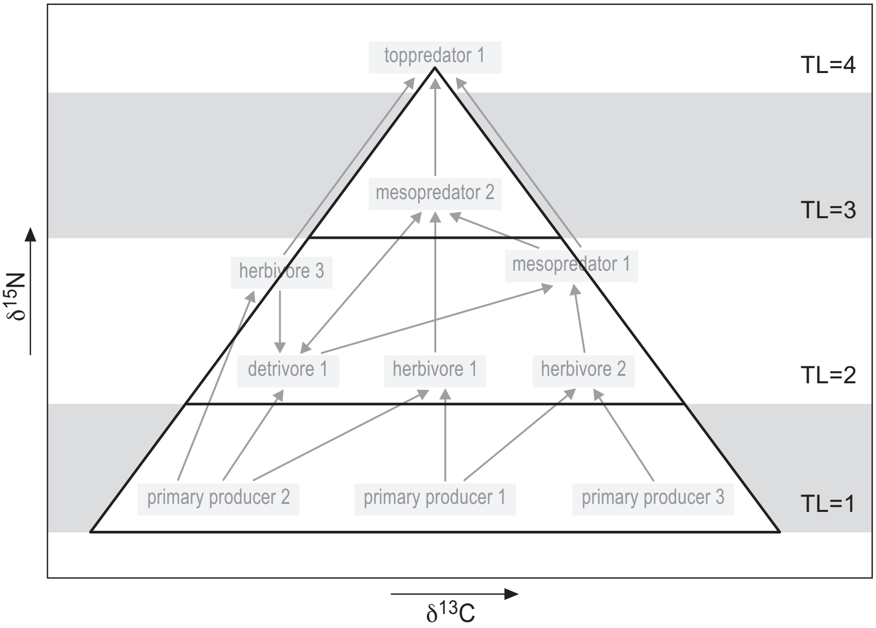


Figure 1.2: A food 'pyramid'.

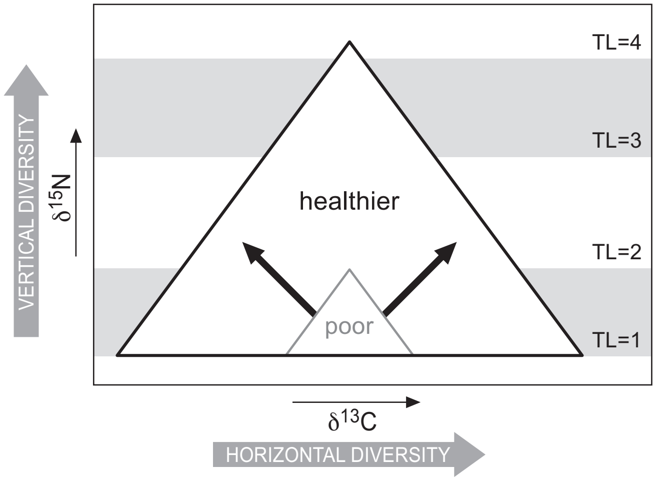


Figure 1.3: Ecosystem health, showing trophic diversity (vertical) and the reliance of sources by the system (horizontal diversity) represented in a stable isotopic bi-plot.

The state of an ecosystem, expressed by the diversity of an ecosystem, can be depicted by a food web structure or food pyramid (Figures 1.2 and 1.3). In such a food web representation, trophic diversity is expressed by vertical diversity, while the range of sources where an ecosystem relies on is expressed by horizontal diversity (Figure 1.3). The horizontal and vertical diversity of a food web structure, together, are indicative for ecosystem health or the state of an ecosystem (Olff et al. 2009). As shown in Figure 1.3, ecosystem health is visible in the size and shape of the contours of the food web structure (here food pyramid) which reflects the diversity of the ecosystem.

Note that, whereas the number of species reflects diversity, the number of feeding links in a food web represents the ecosystem complexity. The horizontal and vertical diversity of a food web structure, thus, does not necessarily describe a complex food web.

The Wadden Sea

The Wadden Sea is one of world's largest intertidal ecosystems along the coast of The Netherlands, Germany and Denmark (Wolff 2000, Eriksson et al. 2010, Hogan & Monosson 2011).

It is fuelled by a multiple sources, internal benthic and pelagic origins and three external sources are together driving the Wadden Sea ecosystem and are distinguishable with help of $\delta^{13}C$ values (Benthic: −16.3 ±0.1SE ‰ $\delta^{13}C$ | Pelagic: −18.8 ±0.1SE ‰ $\delta^{13}C$ | Lake Lauwersmeer: −28.5‰ ±0.3SE ‰ $\delta^{13}C$ | Salt marsh (here island Schiermonnikoog): -28.3‰ ±0.9SE ‰ $\delta^{13}C$). It provides key habitat to approximately 2700 marine species, including the species that connect the Wadden Sea with ecosystems elsewhere on the globe, i.e. the migratory shorebirds (van de Kam, Ens, Piersma et al. 2004, Reise, Baptist, Burbridge et al. 2010).

At first sight, the Wadden Sea might seem rather species-rich, but this is – at least relative to historical references – far from true. Major changes have occurred in Wadden Sea, mostly due to human impact (Lotze, Reise, Worm et al. 2005, Lotze, Lenihan, Bourque et al. 2006). The Wadden Sea area is depleted by more than 90% of formerly important species, more than half of all seagrass and wetland area is destroyed and water quality dropped, while multiple invasive species increased (Lotze 2005, Lotze et al. 2006, Eriksson et al. 2010). Already a 1000 years, the Wadden Sea faced human impact and mild forms of decline, but the last 150 to 300 years human impact escalated and left its traces in forms of steep acceleration of ecological degradation (Lotze et al. 2005, Lotze et al. 2006). The ecosystem changed towards a system with an impoverished food web complexity, which is schematically shown in Table 1.2 (de Jonge, Essink & Boddeke 1993, Wolff 2000, Piersma, Koolhaas, Dekinga et al. 2001, Lotze 2005, Lotze et al. 2005, Lotze et al. 2006, Eriksson et al. 2010). The two most destructive changes that both directly and indirectly impeded the complexity of the Wadden Sea food web were: the disappearance of big top-predators and the strong decline in the important and once so numerously-present ecosystem engineering seagrass meadows and mussel beds at the bottom of the food web, to the point of total loss (Wolff 2000, Polte, Schanz & Asmus 2005, van der Heide, van Nes, Geerling et al. 2007, Eriksson et al. 2010).

Table 1.2:

Period	Change	Cause	Consequence	References
Since ±1100A.D.	Saltmarsh decrease in area and length	Land reclamation, first by monasteries then by farmers and water board RWS	Less silt sedimentation on saltmarsh. Change in saltmarsh vegetation. Higher water turbidity	Dijkema 1987a
Since ±1800	Start large-scale loss of big top-predators (porpoise, ray, shark)	Overfishing	Shift in regulation, from top-down to more bottom-up	Wolff 2000, 2005
Since ±1850	Start sea level rise with ±2 mm/yr in Wadden Sea and North Sea	Climate change, both natural and human induced	Reduced saltmarsh area. Dyke breaches	Behre 2007
1930	Loss Zuiderzee as brackish estuary	Construction of mega causeway Afsluitdijk	Loss of brackish species. Freshwater-marine passage	Dijkema 1987b
1930–1950	Loss of practically all sublittoral (*Zostera marina*) and littoral (*Z. notii*) seagrass meadows	"Wasting disease", combined with construction of Afsluitdijk (increased water turbidity)	Loss of organisms connected to littoral seagrass meadows	Den Hartog 1987, Katwijk & Hermus 2000, van der Heide et al. 2007
Since 1950	Strong increase planktonic primary producers, possibly followed by slight decrease (from 1990 onwards); no information about epi-phytobenthic primary producers	Initial strong increases in eutrophication of surface water, cause by introduction of fertilizers and agriculture, sewage and industrial discharges. Followed by slight decreases (from 1990) due to better water purification	Amplifying the lack of recovery of seagrass meadows due to turbidity of water. Decrease in sight hunting predators	Philippart, Beukema, Cadee et al. 2007
Since 1950	Strong increase fishery intensity. Soft-bottom disturbances	Expansion in power of fisheries fleet	Increase water turbidity caused by soft-bottom disturbance. Increase bycatch. Increase total catch	

Table 1.2: Continued.

Period	Change	Cause	Consequence	References
1965	Start mussel cultivation plots in Wadden Sea (7000ha); harvesting maximal 70-100 million kg/yr (from ca. 1965 onwards), now strongly declined harvest	Disease. Absent spat fall in Zeeland (NL) due to finished Delta Project and diseases	Increase soft-bottom disturbances in sublittoral zone in the Western Wadden Sea	
1969	Loss Lauwerszee as brackish estuary	Construction of the causeway that created lake Lauwersmeer	Loss of brackish species. Freshwater-marine passage	
Since 1980	Rise of mean temperature in The Netherlands, with in total 2 degree/20yrs, a rise that is higher than what is expected based on natural climate cycle	Greenhouse effect, increased emission of CO_2 by increases in fossil fuels	Possibly more predation eg. by shrimp on shellfish because of prolonged predation in winter	Beukema & Dekker 2005, Dekker & Beukema 2007
Since 1983	Strong rise of invasive shellfish, e.g. the Japanese Oyster *Crassostrea gigas* and Razor Clam *Ensis directus*, or Chinese Mitten Crab *Eriocheir sinensis*	Culturing in nursery beds by oyster farmers. Transport with ballast water	Competition between endemic species and introduced invasive species	Nehls, Diederich, Thieltges et al. 2006, Brandt, Wehrmann & Wirtz 2008
1988–1990	Trawling of almost all littoral mussel beds	Absence of spat fall in these years. Lack of mussels	Disappearance of intertidal mussel beds and associated flora and fauna. Decrease of shellfish-eating birds as Oystercatcher, Red Knot and Eider	Beukema & Cadée 1996, Dankers, Brinkman, Meijboom et al. 2001, Wolff 2005
Since 1990	Strong decline of settlement and population size of certain bivalve species, with the ecological extinction of *Macoma balthica* in the Western Wadden Sea around 2000 (a key stone species until then)	Soft-bottom disturbance by cockle-, mussel- and shrimp fishery which reduces settlement- and survival opportunities. Reduction in high-quality prey for birds	Declines in birds that are dependent on *Macoma*, such as Red Knots *Calidris canutus*	van Gils, Spaans, Dekinga et al. 2006

Table 1.2: Continued.

Period	Change	Cause	Consequence	References
Since 2004	Definite closure of mechanical cockle fishery	Protests of nature organisations, based on scientific research	Increased settlement and enforcement shellfish. Slow recovery of benthic fauna	Kraan, Piersma, Dekinga et al. 2007, Compton, Bodnar, Koolhaas et al. 2016
Since 2005	Change of 34 Cockle fishermen from the Wadden Sea to fishing on *Spisula* in the North Sea. Plans to exploitation of Venus clam fishery in Mauritania	Prohibition of cockle fishery in the Wadden Sea	Unknown effects on the coastal strip of the North Sea	
Since 2006	Increase in provisioning permits to hand-cockle fishing	Pressure from fisheries sector, evoking employment and tradition	Decrease standing stock of Cockles (not documented)	
2008	Withdrawal by Council of State of permission of mussel seed fishery in the Wadden Sea	Protests of nature organisations, evoking the European rules and precautionary principle	Less soft-bottom disturbance in the sublittoral. Higher availability of young mussels as food for bivalve-eating birds	
Since 2011	Moderation and regulation of hand cockle fishing	Protests of nature organisations and scientists, demonstrating against degradation of benthic fauna	Increase standing stock of Cockles and indirectly also increases in other benthic species	

Where management in the Wadden Sea in the past focussed on fishery, shipping, coastal protection and recreation, nowadays nature preservation is an important focus as well. Awareness rose that the Wadden Sea is a unique intertidal area, of great natural importance for many natural processes, not in the least because it functions as a crucial staging area for thousands of birds migrating along the East Atlantic flyway. Nowadays, the Wadden Sea is inscribed on the UNESCO World Heritage List and protected by the Ramsar Convention and the bird, water and habitat directives Natura 2000 (Boere & Piersma 2012). Conservation and recovery became key priority for nature management in the Wadden Sea.

Success was booked by prohibition of cockle fishery, manifested by a reviving benthic fauna after several years of recovery, shown by increasing numbers of the bivalve species *Macoma balthica* (Compton et al. 2016). However, despite other restoration efforts – such as reintroduction of the two crucial ecosystem engineers, water quality improvement and recovery for seals – the Wadden Sea is not yet restored to a flourishing system with high complexity and species numbers (Piersma et al. 2001, Eriksson et al. 2010, van der Veer, Koot, Aarts et al. 2011, van Roomen, Laursen, van Turnhout et al. 2012). Key processes that lead to and at the same time indicate the evolvement into a stable and healthy Wadden Sea ecosystem – thus into a 'healthy' and complex state – are thought to be (1) recovery of the two important ecosystem engineering species who had an very important role for the total food web, being the recovery of large fields of (a) stable intertidal blue mussel *Mytilus edulis* beds and (b) sea grass beds *Zostera noltii* and *Z. marina* and (2) the re-appearance of top-predators (Lotze et al. 2005, 2006, Eriksson et al. 2010).

Issues

The shape of the food web structure of the Wadden Sea can unravel the dependency of the sources that drive the Wadden Sea ecosystem, showing for instance the dependence on the particularities of the primary sources of an ecosystem (horizontal diversity). A food web structure can be symmetric showing an equal dependency of all primary producers, or it can be build up skewed with the top of the food web hanging to the left or right, showing the dependence of primary producers in the food web (Figure 1.4). Figure 1.4 presents the theoretical shape of the food web of a marine intertidal system, showing the skewness to the left and right as a respectively pelagic or benthic dominated food web.

The contours of a food web structure can thus determine the flow of energy through an ecosystem and may help us forecast what it means if particular food web elements increase or decrease in numbers or shift in diet. The dependency of primary producers on the above-lying food web, thus the skewness of the contours of the food web structure, can be help to understand (with downstream efforts to improve) the state of the ecosystem. If, for instance, there is an interest in the recovery of toppredators in the system, it is helpful to understand what these toppredators would feed upon and determine what primary producers should drive this energy flow (Figure 1.1 and Figure 1.4). A food web structure can thus help predicting how a system will react to disturbances or restoration (Dunne et al. 2002, Pimm 2002, Catry, Lourenco, Lopes et al. 2016).

The aim and urge to understand and validate the processes that should lead to restoration of the Wadden Sea are supported by scientists, local governments and nature management organisations of national, European and global level. The goals are high, the set time is short. Overall, the main goal is to find and effectuate keys that lead to higher complexity and biodiversity (Eriksson et al. 2010). Characterisation of

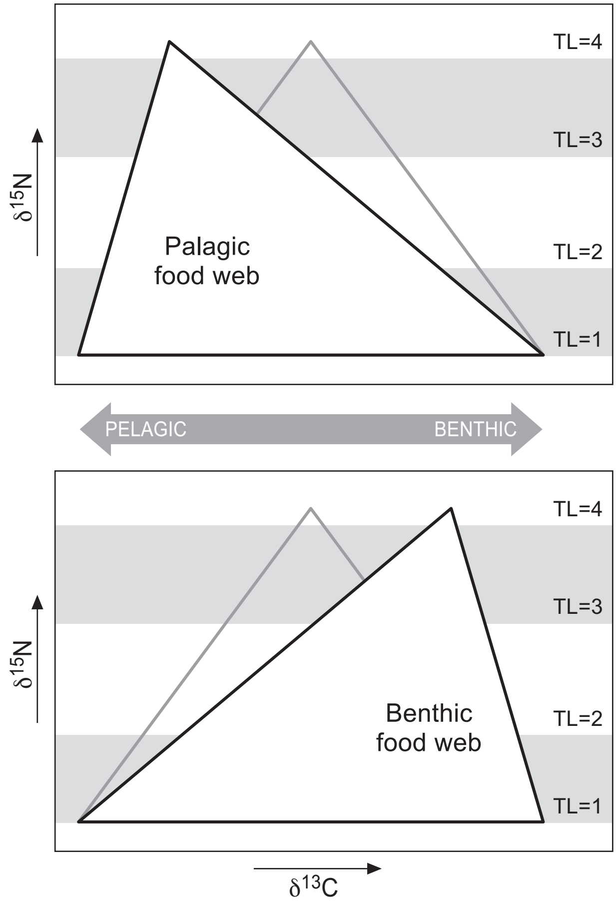


Figure 1.4: Food pyramids for pelagic and benthic food webs.

the Wadden Sea food web would be helpful in finding, effectuating and monitoring the keys to restore the Wadden Sea in its former healthy, flourishing and complex state.

Food web reconstruction could be helpful in following the changes in the Wadden Sea and crucial in timely intervention (Eriksson et al. 2010). Bearing in mind the wish to restore the Wadden Sea ecosystem in its former (pristine) state, if solid information about the food web structure of the former state is lacking, the characterization of a disturbed ecosystem is especially valuable when comparing it to other intertidal ecosystems. Assessing the food web structure of ecosystems creates the ability to compare ecosystems and search for generality of ecosystem health in food web structures.

Quantitative comparison between (the food webs structure of) ecosystems are still rare, while being extremely insightful and thus valuable. As an example, the study of Catry et al. (2016) made a quantitative comparison between the food web structure of four important intertidal ecosystems for migratory birds along the East Atlantic flyway, including Tagus estuary in Portugal, Sidi Moussa in Morocco, Banc d'Arguin in Mauritania and Bijagós archipelago in Guinea-Bissau. Differences and similarities between the structure of these intertidal systems became clear (Catry et al. 2016). The geographically more northern oriented intertidal Wadden Sea ecosystem bordering the Netherlands, Germany and Denmark is similarly comparable to the other four intertidal ecosystems in Catry et al. (2016) (see also the General Discussion of this thesis).

In this thesis I search for the possibility to establish whether we can use indicator species to inform us about the contemporary state and structure of the food web of the Wadden Sea. An indicator species is a strategically chosen species whose state reflects the state of other species in the area, and their mutual trophic relationships. Although the population and survival trends of an indicator species is regularly used to quantify the state of (part of) the ecosystem, we here mainly focus on changes in the food web position of the indicator species, which indicates changes in the part of the food web structure where the indicator species is indicative for.

There are multiple types of indicator species, but here the term 'indicator species' should probably be defined as a 'food web position indicator species' and we assess the food web position of the indicator species by using stable isotopes (Krebs 2001, Carignan & Villard 2002). By studying the food web position of several indicator species, each indicator species can monitor part of the food web and together can give insight in the (changing) state of the total ecosystem. It can help answering how to restore the Wadden Sea and can give insight in the underlying mechanism of the failing or successful conducted restoration attempts. For instance, in the Wadden Sea, a higher trophic indicator species could shift towards more benthic food sources (shown in lower $\delta^{13}C$ values), indicating that (part of) the ecosystem is now more benthic orientated, which infers recovery of the benthic mud flats.

The **research question** that I will therefore try to answer: Can we characterize the spatially variable food webs of the Wadden Sea food web with help of stable isotopes, and is it possible to identify useful indicator species to document future food web changes?

Thesis outline

To demonstrate the power of the approach, **Chapter 2** presents a reconstruction of the food web of a terrestrial part of the entire Wadden Sea ecosystem, the saltmarsh.

Using a unique and well-established ‘chronosequence’ of the Schiermonnikoog saltmarsh, we were able to reconstruct food web changes over time from food web changes over space. We show that baby saltmarshes to a significant extent are externally fed from the marine component of the Wadden Sea ecosystem.

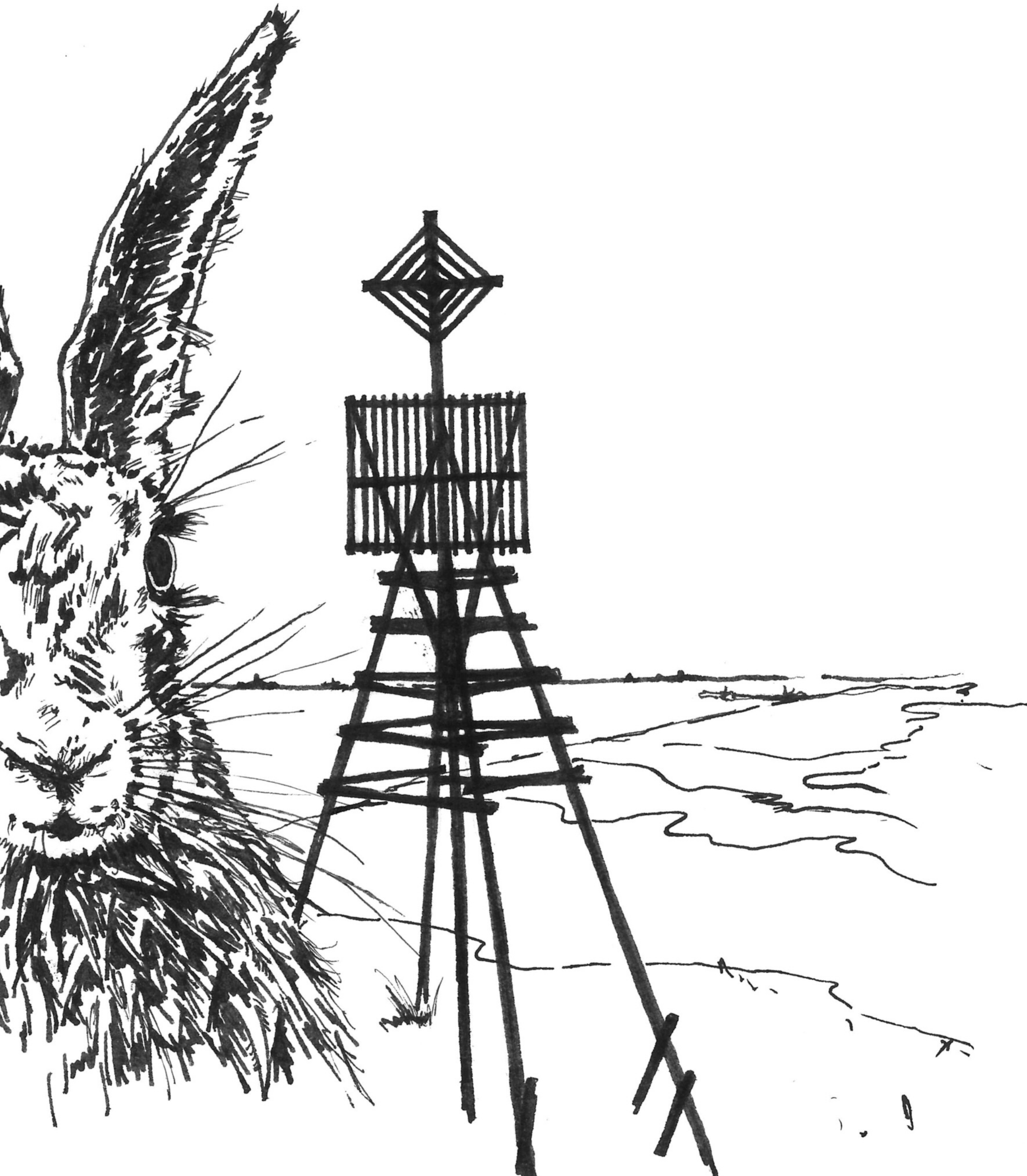
In order to understand the marine Wadden Sea ecosystem, it is valuable to understand how autonomous the Wadden Sea is and whether the system is supported by local benthic, pelagic primary sources or by external sources. Distinguishing between these energy sources is essential for our understanding of ecosystem functioning. In **Chapter 3** we show that the Wadden Sea ecosystem is a primarily internally fuelled, benthic-driven system and show the dependency of benthic sources for species that live in the Wadden Sea, clarifying most of the food web structure on the horizontal axis that represents stable carbon isotopes. The other component of the food web structure, the trophic vertical axis, is highlighted in chapter 4 and 5.

In **Chapter 4** we show that in order to calculate trophic positions it is important to use a justified baseline, acknowledging species specific information such as mobility and primary source (benthic /pelagic) and acknowledge spatial heterogeneity in baselines. Knowing the basic ingredients, we can generate food web structures and assign indicator species for parts of the food web structure that can elucidate potential spatial differences in the state of the ecosystem. Assigning indicator species for this area could help monitoring ecosystem health.

In **Chapter 5** we assess whether Spoonbills are a suitable indicator species. They are, but what for? Rather than being an indicator of the size and composition of the whole food pyramid, Spoonbill diets assessed by stable isotopes (and verified by analyses of regurgitates) are indicator of the presence of small flatfish in the shallow intertidal Wadden Sea

Recalling the connectedness within the entire Wadden Sea ecosystem (Chapter 2), in **Chapter 6** we zoom out to beyond the Wadden Sea and study its international (and indeed intercontinental) connections. We develop an estimation model with which, based on a single blood sample, we can quite accurately assign the temporal occurrence of two subsequent diet switches of individual shorebirds.

In **Chapter 7** I come back to review these results, and conclude that this work has perhaps raised more questions than it did answer. The reconstruction of food webs is more complicated that we had anticipated. For example, the estimations of trophic positions requires information on the spatial heterogeneity in isotope values of the resources and the mobility of the consumers. I conclude that indicators of ‘health’ of the Wadden Sea ecosystem are best represented by the particular functional relationships between resources and consumers which are now relatively well-understood (as for spoonbills, see Chapter 5).



CHAPTER 2

Food web assembly at the landscape scale: Using stable isotopes to reveal changes in trophic structure during succession

Maarten Schrama, Jeltje Jouta, Matty P. Berg & Han Olff

Abstract

Food webs are increasingly evaluated at the landscape scale, accounting for spatial interactions involving different nutrient and energy channels. Also, while long viewed as static, food webs are increasingly seen as dynamic entities that assemble during vegetation succession. The next necessary step is, therefore, to link nutrient flows between ecosystems to local food web assembly processes. In this study, we used a 100-year salt marsh succession in which we investigated the long-term changes in food web organization, especially focusing on the balance between internal versus external nutrient sources. We found that during food web assembly, the importance of internal (terrestrial) nutrient cycling increases at the expense of external (marine) inputs. This change from external to internal nutrient cycling is associated with strong shifts in the basis of energy channels within the food web. In early succession, detritivores are mostly fuelled by marine inputs whereas in later succession they thrive on locally produced plant litter, with consequences for their carnivores. We conclude that this 100 years of food web assembly proceeds by gradual decoupling of terrestrial nutrient cycling from the marine environment, and by associated rearrangements in the herbivore and detritivore energy channels. Food web assembly thus interacts with nutrient and energy flows across ecosystem boundaries.

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Introduction

Food web studies have so far revealed many informative and repeatable patterns in trophic structure (for example, Pimm 1982, Cohen and Briand 1984, Cohen and Newman 1985, Dunne et al. 2002), body size distribution, and topology (Cohen et al. 2003, Woodward et al. 2005). In addition, it is increasingly recognized that the understanding of the architecture of many food webs requires the inclusion of above- and belowground parts of ecosystems, as well as spatial interactions and/or temporal dynamics between food webs (Winemiller and Polis 1996, Moore et al. 2004, Berg and Bengtsson 2007, Rooney et al. 2008). These authors have shown that food webs are composed of different energy channels based on discrete resources, which are often not homogeneously spread in space and time, thus influencing the different components of the food web in their own way. Moreover, spatial interactions among neighbouring ecosystems, such as between ocean and desert, cause some food webs to connect to others on a landscape scale (Winemiller 1990, Polis and Hurd 1995, Rooney et al. 2008). Specifically, spatial subsidies into the detrital part of the food web have been shown to have far-reaching consequences for food web structure and ecosystem functioning. They often lead to higher primary production, which in turn may cascade up trophic chains (Polis and Hurd 1995, Polis and Strong 1996).

Although the role of spatial interactions in food web organizations are increasingly studied, good case studies of food web assembly over sufficient time are still poorly available, especially for food webs that are connected to others at the landscape scale. This lack is possibly explained by the large effort required in the quantification of the food web components in the first place, combined with the additional complications involved in following food web structure over sufficient time. One of the most obvious changes in food web structure that explicitly focuses on this temporal component can be found during primary succession. However, succession research has merely focused on vegetation and soil changes (Clements 1916, Miles and Walton 1993). The few notable exceptions (Van de Koppel et al. 1996, Kaufmann 2001, Neutel et al. 2007) have addressed only parts of food webs, rather than providing an integrated analysis of their structure.

Given the importance of spatial subsidies and temporal changes in food webs for understanding the processes of ecosystem assembly, combining insights from food web ecology on a landscape scale, that is, combining vegetation succession research and food web assembly in single study systems, is now needed. More precisely, we need to find out how different food web components, that is, the green component of food webs, consisting of plants and herbivores and their predators, and the brown component of food webs, consisting of plant litter, detritivores, and their predators interact over a successional sequence by taking the spatial aspects of food webs into account. This can be done by building on recent insights in factors structuring different energy channels in food webs: specifically the balance between the brown (detritivore-driven) and green (herbivore-driven) parts of food webs (Cebrian and Lartigue 2004, Moore et al. 2004, Rooney et al. 2006, Shurin et al. 2006).

In this study, we used a chronosequence reflecting 100 years of primary succession on a coastal salt marsh to study food web assembly on a landscape scale. Previous work on our study system has quantified in detail the dynamics of soil and vegetation succession (Olff et al. 1997) and its interaction with vertebrate herbivores (Van de Koppel et al. 1996, Van Wijnen and Bakker 1999, Kuijper and Bakker 2005). This chronosequence has been carefully validated by 35 years of study of permanent plots, which has justified its space-for-time replacement (van Wijnen et al. 1997, Schrama et al. 2012). This chronosequence approach allowed us to study 100 years of successional dynamics during the same year. We now add analyses of the trophic dynamics, by explicitly focusing on the brown and green part of the food web along the chronosequence to study changes in food web organization over succession. In addition, we use stable isotope analyses to study the landscape origin of the carbon and nitrogen used by different compartments.

Stable isotopes are excellent tools to study temporal changes in food web organization, and nutrient and carbon sources for the different energy channels, especially in coastal environments. Carbon isotopes are useful in discriminating marine from terrestrial sources (Polis and Hurd 1995, Maron et al. 2006), especially when the terrestrial plant species are predominantly C3 plants, which is the case in our system. Nitrogen isotopes are useful in studying the trophic position of different groups (Hobson and Welch 1992, Post 2002), and can thus also be used to study changes in trophic position and structure throughout succession. Specifically, we unravel which nutrient and energy sources fuel the food web in the various stages of vegetation succession. Then we study how the main food web organization in this ecosystem changes over 100 years of primary succession. For this, we combine information on animal diets with measured nitrogen isotope values for each main trophic group in different stages of succession.

Methods

Study Area and Sampling Design

Our study area was located on the island of Schiermonnikoog (53°30'N, 6°10'E), The Netherlands. M. Schrama et al. A sequence of vegetation succession stages on the salt marsh was present, where the earliest stages are formed on the east side of the island, and later stages of succession were situated 8 km to the west (Olff et al. 1997). The sampling locations get inundated regularly at high tides. Marine algae and other

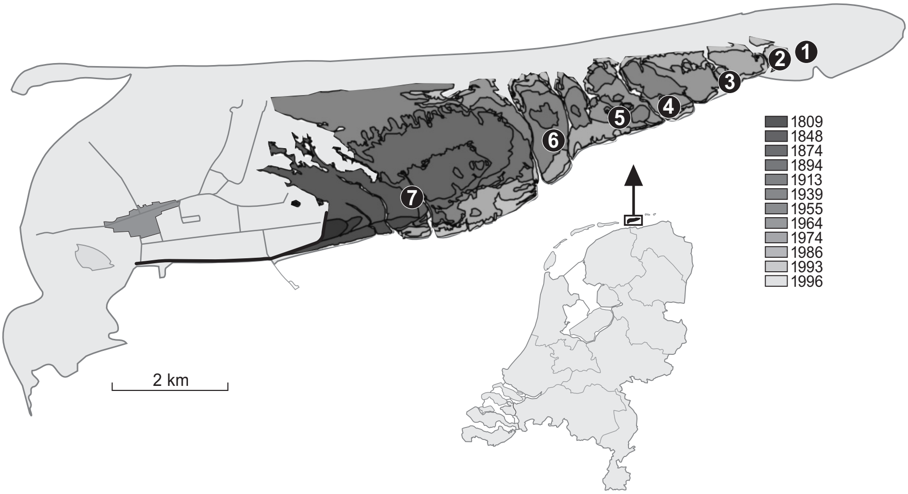


Figure 2.1: Map of the study area on the island of Schiermonnikoog, the Netherlands. Numbers 1–7 refer to the age (in 2010) of the seven study sites in the different successional stages: (1) 0 years, (2) 10 years, (3) 25 years, (4) 35 years, (5) 45 years, (6) 55 years, (7) 100 years in 2010. Different isoclines represent the different stages of vegetation colonization (until 1997). The chronosequence map was constructed using topographical maps and aerial pictures (methods described in Olff and others 1997; De Jager 2006).

organic material wash ashore during these inundations. At high tide, many resting waders are present in the area, especially during spring and autumn migration, but many non-migrating waders are also present during the whole year.

Seven succession stages were identified, estimated as 0, 10, 25, 35, 45, 55, and 100 years of primary succession in 2010 (Figure 2.1, see Olff et al. 1997 for details). Salt marsh age at each successional stage was estimated from topographic maps, aerial photographs, and the thickness of the sediment layer accumulated on top of the underlying sand layer (Olff et al. 1997, Van Wijnen and Bakker 1999), and calibrated using long-term observations of permanent plots (van Wijnen et al. 1997). The sites were selected to have a similar base elevation (vertical position with respect to mean sea level at the initial elevation gradient on the bare sand flats, before additional sedimentation happened due to vegetation succession). A base elevation of 1.16 m (± SE 2.2 cm) above Dutch Ordnance Level (N.A.P.) was used to select the sites for this study. Details on every sampling site can be found in the electronic appendix, Table A2.1. Along our succession gradient, inundation frequency declines due to ongoing sedimentation. This is associated with a gradual decline in the clay and organic matter input along the successional sequence (Olff et al. 1997). The earliest stage of succession had an average inundation frequency of 184 times y^{-1} (26% of all tidal cycles), whereas the last stage of succession has an inundation frequency of 131 times y^{-1} (18% of all tidal cycles).

At every succession stage (site) five 25-m spaced plots (5 m × 5 m each) were placed in which the main trophic groups from the green and brown part of the food web were sampled. Between April 23 and May 14, 2010, field collections of all dominant animals, plants, and marine deposits (species that represent >90% of the biomass in their respective trophic group) were done in each of these plots. The changes in abundances of the different species, and their aggregations into trophic groups, are reported in a previous study (Schrama et al. 2012). Sampling methods for all trophic groups for the stable isotope analysis are described in the following paragraphs. For all details on the dominant species that were collected and sample size for every species or organic matter source, see electronic appendix, Table A2.2.

Estimations of Marine Input

Both bird guano and macro-algae that drift ashore can be an important input of nutrients (Polis et al. 1997, Maron et al. 2006) and estimations were made of the input of both sources of nutrients. Macro-algal dry weight was estimated in March and April 2010 by collecting all macro-algae from each 5 m × 5 m plot, which were subsequently rinsed, and dried at 70°C for 48 h. Afterwards, samples were weighed to determine dry weight per square meter. Bird densities were estimated by weekly counts of the numbers of resting birds at all sampling sites during the months March and April. Every location was visited four times. At every site, we used one 1-ha plot in which the total number of resting birds per species was estimated between 1 h before and 3 h after high tide. Per species, the total number of bird minutes was calculated by multiplying the number of resting birds at every location times the number of minutes spend in each of the hectare plots.

Sampling for Isotope Analysis

Terrestrial Plants

At each plot location, we collected ten leafs from ten different individual plants for each of the dominant plant species. The dominant plant species comprised at least 90% of the locally produced biomass. To standardize samples, only fresh plant leaves were collected. Stable isotope analysis of plant leaves were done on samples from succession stages 0, 10, 45, and 100 years, because we expected no strong differences in isotopic signals between sites. At each plot, a sample of local litter material was collected by taking five random samples of 10 g of dead local vegetation, which were not overgrown with algae or covered in clay. These samples were well-mixed in a bowl from which a subsample of about 5 g was taken for analysis.

Marine POM

Because no flooding events occurred during the sampling period, we sampled marine particulate organic matter (POM) at high tide at a distance of 150 m south of succession stages 10, 45, and 100 years. At five points per site, spaced 25 m apart, we used a plastic hand-held net (30 cm 9 30 cm) with fine mesh (500 lm) which was manually moved five times over a distance of a meter through the upper 50 cm of the water column to catch drifting material. The content of the net was deposited on a clean plastic sheet and funnelled into a 2-ml plastic tube with demi water and stored at −20°C. Because we expected no large differences for marine POM stable isotope signals for both $\delta^{13}C$ and $\delta^{15}N$ between sampling locations, no additional sampling was done in other succession stages.

Diatoms

As marine diatoms represent a significant fraction of the organic matter that is deposited during floodings (Boschker et al. 1999), we made collections of diatoms at locations close to the plots. Diatoms were collected from the marine sediment at low tide at a distance of 150 m south of succession stages 0, 10, 45, and 100 years. The five sampling plots per site were spaced 25 m apart. From each of the five samples per site, we took one sediment sample of 10 cm × 10 cm × 1 cm deep (100 cm^3) from which diatoms were extracted in the laboratory. To separate the diatoms from the sediment, we applied the 'lens-tissue method' (Eaton and Moss 1966). After the diatoms migrated onto a GF/F Whatman filter (average pore size = 0.7 lm), the content was deposited on a plastic sheet and funnelled into a 2-ml plastic tube using demineralized water and stored at −20°C. As we expected no strong differences between sites for both $\delta^{13}C$ and $\delta^{15}N$ isotope signals, we did not sample any of the other sites.

Marine Macro-algae

We collected the macro-algae *Fucus* spp. Because this species comprises the majority of the deposited marine macro-algae (>90%, see Table 2.1). Samples were collected in all 9 5 m plots at succession stages 0, 10, 45, and 100 years. Every sample contained five random leaves of individual *Fucus* spp. deposits, which were at least 2 m apart. After collection, all samples were brought to the laboratory where they were carefully rinsed with demineralized water to remove other organic material and mineral sediments and stored in 2 ml at −20°C.

Invertebrates

Dominant invertebrate species (identified as dominants in Schrama et al. (2012)) were collected in all subplots, using a modified leaf blower (Echo Shred 'N' Vac, net build inside with a mesh size of 1 mm). After applying the leaf blower for 2 min in each 5 m × 5 m plot, the net was emptied in a white plastic container and living specimens were collected by hand and put into 2 ml plastic tubes. In addition to sampling with the leaf blower, we used hand collections to sample the amphipod *Orchestia gammarellus*, Enchytraeds and the snail *Ovatella myositis*. Each sampling tube contained at least four individuals of each species. After collection, all tubes were stored at −20°C. Literature and personal observations on feeding preference were used to assign species to different trophic groups: herbivores, detritivores, herbivore-feeding carnivores, and carnivores feeding on both herbivores and detritivores (hereafter called omnivorous carnivores). In total, 10 species of invertebrate herbivores were collected, but not the same species from all succession stages. In total 14 species of carnivores were collected. The group of carnivores feeding on herbivores and detritivores contained mostly spiders (Erigonidae and Lycosidae), beetles (Carabidae) and ants (Formicidae).

Table 2.1: Resting marine birds (in bird min h^{-1} ± SD) and amount of marine macro-algal input (mg m^{-2} $month^{-1}$) in each of the stages of succession.

	Number of bird minutes per hour per hectare succession stage (years)													
Bird name	**0**	SD	**10**	SD	**25**	SD	**35**	SD	**45**	SD	**55**	SD	**100**	SD
Oystercatcher (*Haematopus ostralegus*)	32,572	30,840	40	44	0	0	0	0	0	0	0	0	0	0
Dunlin (*Calidris alpina*)	1,800	2,080	692	760	0	0	0	0	0	0	0	0	0	0
Brent goose (*Branta bernicla*)	452	520	420	460	0	0	0	0	0	0	0	0	0	0
Herring gull (*Larus argentatus*)	152	172	20	20	0	0	0	0	0	0	16	16	0	0
Black-headed gull (*Chroicocephalus ridibundus*)	60	68	0	0	0	0	0	0	0	0	0	0	0	0
Eider duck (*Somateria mollissima*)	0	0	360	396	0	0	0	0	0	0	0	0	0	0
Grey plover (*Pluvialis squatarola*)	0	0	20	20	0	0	0	0	0	0	220	200	0	0
Lesser black-backed gull (*Larus fuscus*)	0	0	40	44	0	0	0	0	0	0	0	0	0	0
Redshank (*Tringa totanus*)	0	0	32	36	0	0	0	0	0	0	0	0	0	0
Total marine birds	*32,572*	*30,840*	*1,624*	*1,304*	*0*	*0*	*0*	*0*	*0*	*0*	*236*	*216*	*0*	*0*
Marine macro-algae (mg m^{-2} $month^{-1}$)	594	223	797	196	5	3	2	1	2	0	3	0	6	1

Bird minutes were calculated by multiplying the number of birds times the number of minutes birds spend in each hourly observation in each hectare plot.

Carnivores that fed on herbivores were different species of ladybugs (Coccinellidae) and parasitoid wasps (Ichneumonidae). In total 10 species of detritivores were collected, mainly Amphipoda, Isopoda, and beetles. Because many detritivore species cover the whole spectrum of food sources and because little is known about their exact feeding preference, no a priori subdivision of this group was made.

Marine Birds

Because oystercatchers (*Haematopus ostralegus*) and lesser black-backed (*Larus fuscus*) were found to be dominant roosting birds at the first successional stage, five samples of fresh feces of both bird species were collected in five 25-m spaced 3 m × 3 m plots. These plots were located 50 m south of the plots at which the other collections were done, but only at the first successional stage. Per species per sample we took one individual pellet of guano from 5 individual birds, directly after defecation, using a pair of tweezers.

Stable isotope analysis

All invertebrate, plants, and marine macro-algae samples were stored frozen at –20°C and processed by freeze-drying and grinding with a pebble mill (1000 rotations per minute for 2 min using a Retsch MM2). The δ^{13}C and δ^{15}N isotopes values were determined by using a Thermo Flash 2000 elemental analyser coupled to a Thermo Delta V isotope ratio mass spectrometer. Isotope values were calibrated to a laboratory acetanilide standard (δ^{13}C –26.1 ‰ and δ^{15}N 1.3 ‰ calibrated on NBS-22 and IAEA-N1, respectively) and corrected for blank contribution. The samples were mostly analysed in duplicate and the reported data represent the mean of these analyses. The results are reported on the per mille scale with respect to Vienna Pee Dee Belemnite (VPDB) and graphically presented in δ^{13}C and δ^{15}N isotopes diagrams, to visualize changes in carbon and nutrient sources and trophic structure during succession. The results were graphically presented in isotope ratio diagrams to analyse changes in carbon and nutrient sources and trophic structure during succession.

Statistics

For the analysis of changes in food web stable isotopes composition estimations for species were averaged within trophic groups. To test for differences between trophic groups or within trophic groups between succession stages, general linear models with post hoc Tukey HSD tests were done as assumptions needed for doing parametric analysis were met. Linear least square regressions were done within each trophic group to find changes in stable isotope ratios for both nitrogen and carbon over succession, using Statistica 9.0.

Results

Food Web Composition

Plotting all species in the various plant successional stages in a δ¹⁵N – δ¹³C plane reveals major changes in food web structure (Figure 2.2A–D). A strong difference between δ¹³C values of salt marsh plants and marine diatoms was observed, of about 10‰ of δ¹³C on average. On average herbivores had 2–3 ‰ higher δ¹⁵N values than primary producers (Figure 2.2A, C). Also, the δ¹⁵N values for carnivores were 2–3 ‰ above values for herbivores and 3–4 ‰ above detritivores, except in the first stage of succession.

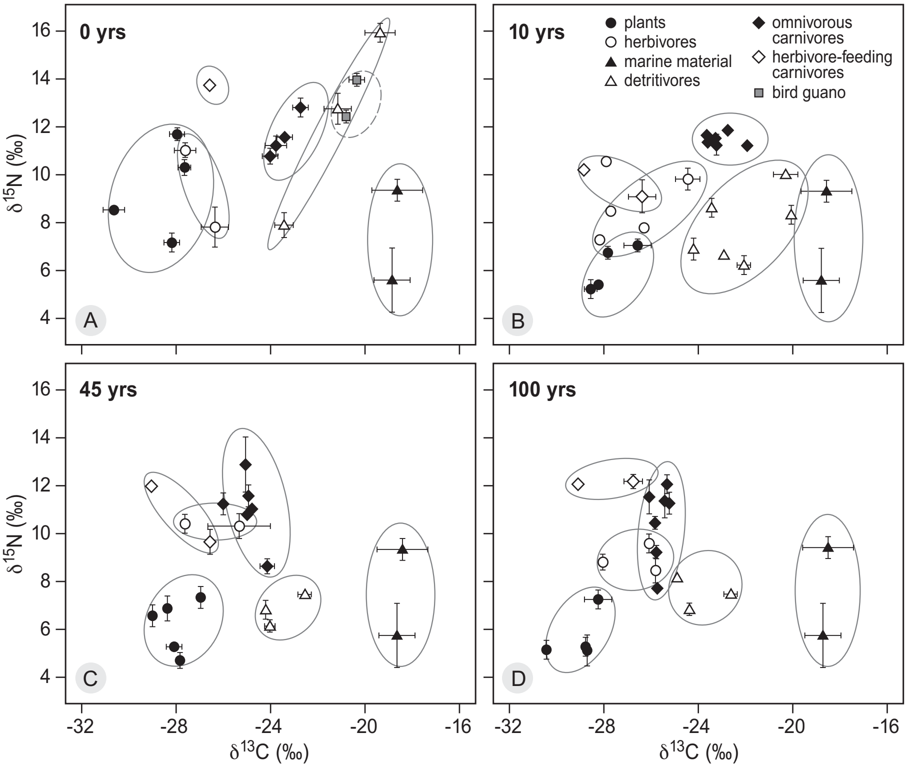


Figure 2.2: All species in a C–N plane depicted in four succession stages: A) 0 years, B) 10 years, C) 45 years, and D) 100 years. Circles with different shadings represent different trophic groups, indicated in the legend. Note that primary producers show only very small shifts on the carbon-axis over time. Mark the widening gap between marine primary producers and other trophic groups (particularly carnivores and detritivores) over successional time.

From Early to Intermediate Succession

To obtain insight on how various trophic groups that either belong to the green web (terrestrial plants and herbivores) or the brown web (detritus and detritivores) are fuelled during early succession and how this changes towards intermediate succession stages, we graphed trends of average $\delta^{13}C$ signatures (Figure 2.3, mostly reflecting changes in base levels) and $\delta^{15}N$ (Figure 2.4, mostly reflecting trophic changes) over time. Differences between $\delta^{13}C$ levels of the green web and the brown web were greatest during early succession (Figure 2.3A–D).

The low carbon isotopic value for detritivores (-21.2 ‰ ± 0.42) at the start of succession is much lower than for herbivores (-27.4 ‰ ± 0.21; Tukey HSD; n = 5; P = 0.002) and herbivorous carnivores (–26.0 ‰ ± 0.9; Tukey HSD; n = 5; P < 0.05). This suggests that detritivores derived their energy initially mostly from marine sources.

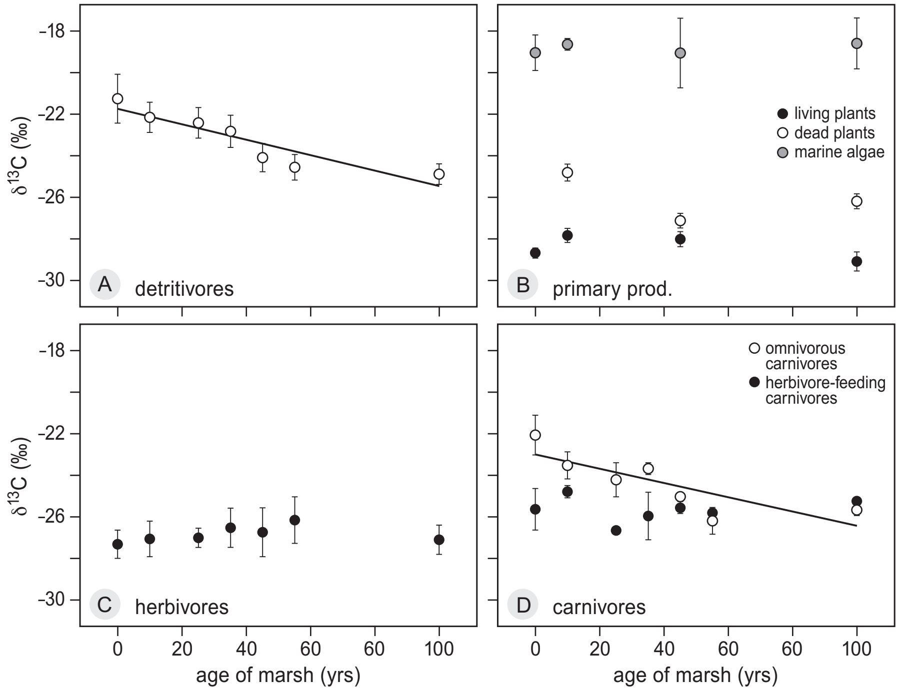


Figure 2.3: δ($^{13}C/^{12}C$) values for all trophic groups. Symbols show averages ± SE. Groups belonging to the brown web are depicted with open circles; groups belonging to the green web are depicted with black circles. A) Detritivores (linear regression: $R^2 = 0.41$; $P < 0.001$), B) marine and terrestrial primary producers, C) herbivorous invertebrates, D) herbivore-feeding carnivores and carnivores feeding both on herbivores and detritivores (linear regression: $R^2 = 0.66$; $P = 0.02$).

Marine sources such as diatoms, bird guano, and marine macro algae had average δ^{13}C levels between −21 and −18 ‰ and were most abundant in early succession. Numbers of resting marine birds and input of marine macro algae (isotope value) were highest in the first two stages of succession (Table 2.1), with oystercatchers (*Haematopus ostralegus*) making up the majority of resting birds. Interestingly, omnivorous carnivores (that is, feeding on herbivores, detritivores, and other carnivores, such as spiders) had similar elevated levels of δ^{13}C to those of detritivores (−21.2 ‰ ± 0.42 vs −23.0 ‰ ± 0.52, Tukey HSD; n = 5; P > 0.5), whereas herbivore-feeding carnivores, such as ladybugs resembled herbivore δ^{13}C-values in these early stages of succession (−27.4 ‰ ± 0.21 vs −26.0 ‰ ± 0.9; Tukey HSD; n = 5; P > 0.5). This suggests that the omnivorous carnivores were mostly feeding on the marine subsidized detritivores during this stage of succession.

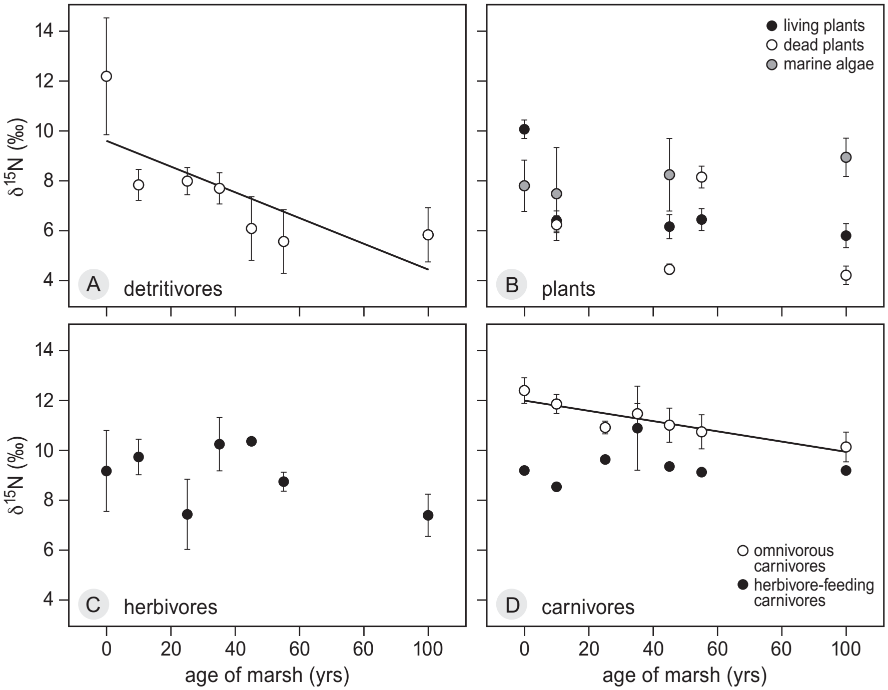


Figure 2.4: $\delta(^{15}N/^{14}N)$ values for all four trophic groups. Symbols show averages ± SEM. A) Detritivores (linear regression: $R^2 = 0.48$; $P < 0.05$), B) terrestrial and marine primary producers, C) herbivores, D) carnivores feeding on herbivores and carnivores feeding both on herbivores and detritivores (linear regression: $R^2 = 0.81$; $P < 0.005$). Note the high value for δ^{15}N in the first succession stage for both A and B.

We found very high $\delta^{15}N$ values for detritivores ($\delta^{15}N$ 12.5 ‰ ± 0.2) during the first stage of succession (Figure 2.4A–D), which were especially high for Enchytraeds ($\delta^{15}N$ levels between 15.2 and 16.5, Figure 2.2A). This again suggests that at least some dominant species in this trophic group feed on high trophic marine sources ($\delta^{15}N$ values 5.6 ‰ ± 1.1 for macro-algae and 8.6 ‰ ± 0.2 for diatoms vs 13.0 ‰ ± 0.3 for bird guano). Omnivorous carnivores ($\delta^{15}N$ 11.5 ‰ ± 0.2) also yielded high levels of $\delta^{15}N$ during early succession, indicating again a trophic link between omnivorous carnivores and the detritivore part of the web. Interestingly, the few terrestrial plant species that we sampled in the earliest successional stage also had slightly elevated levels of $\delta^{15}N$ compared to the same plant species in the next stage of succession (Tukey HSD; $n = 5$; $P < 0.001$; Figure 2.4B), which indicates that their nitrogen may have come from marine sources.

From Intermediate to Late Succession

For herbivores, we observed no change in $\delta^{13}C$ between early and late successional stages, whereas detritivores and omnivorous carnivores showed a strong decrease in $\delta^{13}C$ isotopic signal (Detritivores: $R^2 = 0.41$, $P < 0.001$, Figure 2.3A; Omnivorous carnivores: $R^2 = 0.66$, $P = 0.02$, Figure 2.3D). Patterns for $\delta^{15}N$ were similar, but somewhat less pronounced. Both detritivores and omnivorous carnivores showed a gradual and significant decrease in $\delta^{15}N$ levels over succession (Detritivores: $R^2 = 0.48$, $P < 0.05$, Figure 2.4A; Omnivorous carnivores: $R^2 = 0.81$, $P < 0.005$, Figure 2.4D), whereas herbivores and herbivore-feeding carnivores remained stable over succession (Figure 2.4C, D). Both marine and terrestrial primary producers had the same $\delta^{15}N$ signal in the later stages of succession. The clear observed differences in $\delta^{13}C$ signal between herbivores and detritivores during early succession disappeared towards later successional stages. This was accompanied by a gradual replacement in detritivore species. Although in early succession *Fucellia maritima* and Enchtraeid worms are dominant, beach hoppers (*Orchesia gammarellus*) comprise most of the detritivore biomass in later stages of succession.

Discussion

Our results strongly suggest that during this primary succession over a period of more than 100 years, the energy and nutrient sources fuelling this terrestrial food web changed from mostly driven by external, marine inputs towards a dependence on internal nutrient cycling (Figure 2.5). Both macro-detritivores and omnivorous carnivorous invertebrates (that is, the brown food web component) showed a remarkable shift in $\delta^{13}C$ and $\delta^{15}N$ isotope values with succession, whereas other components

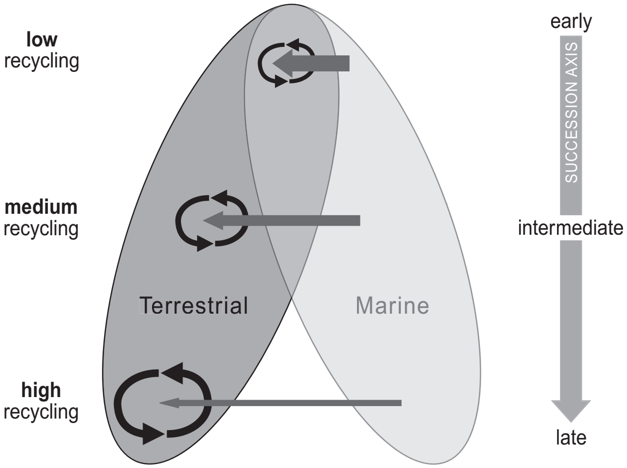


Figure 2.5: Conceptual overview of changes in nutrient supply to the food web over a gradient of successional stages. The two circles depict two ecosystems: the intertidal marine and the terrestrial salt marsh ecosystem. Dark grey arrows indicate the magnitude of nutrient flow from the marine towards the terrestrial ecosystem and circular black arrows indicate the magnitude of local nutrient cycling. We distinguish three distinct phases during succession: early, intermediate, and late succession. In early succession, external (marine) input of nutrients supports a brown web (detritus-detritivore dominated, indicated by a thick grey straight arrow from marine to terrestrial) with low internal production. Intermediate succession is characterized by lower external input of nutrients and a fairly high local production, which supports both a green (plant-herbivore dominated) and a brown web. Late succession is characterized by low external input of nutrients, high internal cycling and low quality plant material, which results in a brown-web dominated state.

of the food web, such as plants and their herbivores (that is, the green web food component) remained more similar in this respect. This pattern is likely caused by both a decline in marine inputs during succession and an increase in nutrient pools and nitrogen mineralization due to the development of a litter layer as observed in earlier work (Van Wijnen and Bakker 1999). So, the role of the brown web along this successional chronosequence changes from being vectors of external nutrients in early succession towards agents of internal cycling in late succession, whereas the green part of the food web remains dependent on local production all along the successional gradient. Several other studies have shown that the successional dynamics on Schiermonnikoog are highly comparable to those on other natural salt marshes in North Western Europe (Bakker et al. 1993, De Leeuw et al. 1993, Olff et al. 1997, Kuijper et al. 2003). This suggests that our results on the food web dynamics in the different stages of succession are likely to represent a general pattern and that similar patterns in food web assembly can be found on other natural marshes. Recently, it was shown that the contribution of marine carbon in the diets of invertebrates declines across the sea-land axis in dune ecosystems (Colombini et al. 2011), which is another indication that this may be a general pattern for many more ecosystems.

Moreover, we expect that our sampling along the chronosequence accurately captured the temporal patterns of the food webs dynamics. We expect that measuring in a different season would not change the qualitative nature of the results because (a) the signal for the stable isotopes in the tissues of invertebrates yields an integrated account of the feeding patterns, which gives a much better temporal integration than for instance gut content analysis (Post 2002); (b) most species only have one generation time per year with a pronounced peak in July, owing to a relatively short growing season (Irmler and Heydemann 1986). It is nevertheless conceivable that the marine signal for species in the brown web would be stronger if samples were collected shortly after a flooding event (or in winter). However, this would only change the results in a quantitative way because we would still expect the highest relative marine input in the earliest successional stages. We therefore expect that our main conclusions will hold upon more detailed analysis at other locations, in other ecosystems and in different seasons.

What Causes Succession to Start?

The main sources of energy and nutrients for the food web assembly in the first stage of succession shows many similarities to the Baja California islands that were described by Polis and Hurd (1995). Both have a high marine input, a low local primary production and a high abundance of invertebrates that do not rely on locally produced organic material, but rather on detritus. Peak standing biomass of living vegetation in our earliest stage varies between 5 and 50 g m^{-2} and covers only up to 2% of the soil surface, whereas in later stages of succession peak standing biomass is between 830 and 1050 g m^{-2} (Schrama et al. 2012). A difference, however, between our first succession stage and the desert islands described by Polis and Hurd (1995), is the much higher rainfall and nutrient inputs by inundations and guano from waders at our study site, which allows the onset of long-term succession towards dense vegetation.

Therefore, we hypothesize that nutrients that enter in the first stage of succession operate as a ‘kick-start’ to long-term food web assembly. Which of the different components of marine subsidy, that is, guano, macro-algae, diatoms, or carrion is the most important source for fuelling the food web at this stage of succession was not the focus of our study. However, our bird count data suggest that nutrients from bird guano may play a very important role in starting vegetation succession and associated food web assembly. Using data from Zwarts andBlomert (1996) on fecal nutrient concentrations of marine birds, combined with our observed densities, we can roughly estimate that the first stages of succession may receive up to 30 kg of N per ha per year, whereas macro algae yield only 8–10 kg N ha^{-1} y^{-2}. As the main bird species responsible for this are non-migratory oystercatchers, this input is relatively constant throughout the year, not limited to specific seasons. Furthermore, this nitrogen input by high trophic level marine-feeding predators provides a good explanation for the relatively heavy isotope signals that we found for early successional plants and detritivores. So, we conclude that our earliest successional stages can be seen as marine-subsidized food webs, both at the trophic level of the primary producers as well as on higher trophic levels.

Causes of Declining Marine Inputs

The suggested reduction of marine inputs towards later successional stages is likely caused by a combination of factors. Firstly, waders and gulls have high tide roosts in the sparsely vegetated zones that surround the mudflats, where the primary successional stages are also located (pers obs. MJJS, MPB, Rogers 2003). Secondly, a 'sieving effect' of taller vegetation in older stages at the direct edge of the salt marsh prevents the sedimentation of marine material onto the marsh at high tides, where larger organic matter such as macro-algae are sieved out first (Temmerman et al. 2005). However, this may imply that our estimation of the deposition of marine subsidy provides an underestimation of the total amount of marine subsidy, especially of the small organic matter fraction. This unknown POM fraction may be more important for explaining successional food web dynamics than we originally anticipated, especially in the intermediate successional stages (up to 35 years). Two lines of argument support this hypothesis. First, the thickness of the sediment layer (which is a mix of clay particles and small POM (Olff et al. 1997)) increases steeply towards the 35- to 45-year-old stage (Appendix, Table A2.1), after which it levels off. Secondly, we find that the $\delta^{13}C$-signature for both detritivores and carnivores shows a less strong marine signal after 35–45 years of succession. To what extent small organic matter is indeed more important in sustaining the (brown) web than the larger fractions of organic material deserves further attention.

The consequences of the observed decrease in external nutrient inputs of marine origin are most clearly observed in the isotopic signal for detritivores and omnivorous carnivores. Although in early succession these groups exhibit a strong external (marine) signal, the diminishing external marine input – not only in relative but also in absolute terms – causes the brown part of the food web to rely more on the local production of plant litter as succession proceeds. Local plant biomass production is high in late successional stages and so is local litter input (Schrama et al. 2012, Olff et al. 1997). Detritivore species now have isotopic signals similar to plants, carnivores, and herbivore species, which indicates that both the green and the brown part of the food web mainly use terrestrially produced organic matter as a source of nutrients. The increase in N mineralization towards later successional stages is driven by the enhanced total pool of soil nutrients which accumulates during succession due to vegetation development, a cumulative effect of more nutrient inputs than losses during all stages (Olff et al. 1997, Van Wijnen and Bakker 1999). Therefore, we conclude that the combined decrease in the input of marine, external organic material and an increase of the primary production causes both the green and brown parts of the food web to become increasingly 'fuelled' by local primary production towards late successional stages.

The Role of Carnivorous and Detritivorous Invertebrates in Early Succession

From other studies on primary succession it is known that spiders and ground beetles are amongst the first carnivorous species to invade an area (Hodkinson et al. 2001, Kaufmann 2001, Coulson et al. 2003). We also observe this in our salt marsh food web assembly, where omnivorous carnivores and detritivores thrive on external inputs in the earliest successional stages. On early successions at glacier forelands and volcanic sediments, external inputs of windblown insects are also an important organic source for early food web assembly (Edwards and Sugg 1993, Coulson et al. 2003). This will also happen in our system, but we suggest that on salt marshes the importance of this input is minor compared to marine nutrient inputs. Although the relative importance of local primary production versus the external input of energy and nutrients as a food source for these early soil dwelling carnivores and detritivores has seldom been addressed, our findings emphasize the importance of external marine inputs of nutrients and energy to the first stage of succession. This upsets the idea further that colonizing plants are required to 'get nutrient cycling and vegetation succession going'.

Consequences of Declining Detritus Quality

The quality of the coarse detritus that is decomposed by the brown part of the food web in early succession is likely much higher than the later stages (Olff et al. 1997), even though both stages of succession are dominated by members of the brown web (Schrama et al. 2012). Enchytraed worms and *Fucellia maritima* are dominant species in the earliest succession stage, where the high deposition of marine derive organic material is likely to be of higher quality than the terrestrial detritus in the later successional stages (Shurin et al. 2006). The intermediate to later stages of succession have increasingly larger stocks of decreasing quality of organic matter (Van der Wal et al. 2000, Kuijper et al. 2004). This decline in litter quality is most likely driven by increasing importance of light competition among plants, which results in dominance of grass species with high investments in structural tissues of the dominant plant species (Huisman and Olff 1998) and resulting low litter quality (Olff et al. 1997). The macro-detritivores in this part of the salt marsh consist almost entirely of the species *Orchesia gammarellus*, a semi-terrestrial amphipod. Its litter processing as well as its digging behaviour make litter more easily accessible and decomposable for bacteria and fungi (Moore and Francis 1986). We suggest that because of the lower quality of litter in late succession, litter pre-processing by these macro-detritivores may be essential for nutrient mineralization. So, where the first stage of succession resembles a marine food web, with high quality organic material, high turnover of organic matter and low standing plant biomass (Cebrian and Lartigue 2004, Shurin et al. 2006), it gradually changes into a typical terrestrial food web with lower organic matter quality, slower cycling of nutrients and higher standing plant biomass.

Conclusions

We suggest that our findings represent a general pattern of ecosystem assembly, which is schematically represented in Figure 2.5. The earliest successional stages are subsidized with external, high quality organic material which 'kick starts' early successional vegetation development. After the initial kick-start, internal cycling of nutrients becomes progressively more important, where the brown part of the food web increasingly depends on this internal cycling of nutrients. The amount of subsidy of organic matter will determine the subsequent rate of food web assembly. From several other ecosystems it is known that primary succession is happening much slower, which is likely caused by lower initial inputs. However, several recent studies on primary succession (Sugg and Edwards 1998, Kaufmann 2001, Hodkinson et al. 2004), now seem to agree on the importance of some form of external input of nutrients to the system to initiate long-term developments. For our ecosystem we conclude that 100 years of food web assembly proceeds by gradual decoupling of terrestrial nutrient cycling from the marine environment, by associated rearrangements in energy channels between the brown and green part of food webs, and increasing importance of macro-detritivores for nutrient cycling during later successional stages. Food web assembly thus interacts with nutrient and energy flows across ecosystem boundaries.

Appendices

Table A1: General characteristics for each of the sampling sites.

Age succ. stage (yrs)	Elevation cm above NAP (±SE)	Flooding freq. yearly average 1998–2008 (±SE)*	Sediment layer thickness cm (±SE)	Vegetation height cm (±SE)
0	115.2 (2.3)	184.2 (5.8)	0.0 (0.0)	1.2 (0.3)
10	116.2 (2.0)	178.3 (5.9)	4.9 (0.3)	5.9 (0.7)
25	116.0 (3.2)	178.3 (5.9)	7.2 (0.2)	10.2 (1.3)
35	118.4 (1.9)	164.8 (6.5)	13.5 (0.6)	8.1 (1.7)
45	123.4 (1.9)	136.9 (6.5)	15.3 (0.2)	9.4 (0.8)
55	125.0 (2.1)	127.7 (7.0)	14.4 (0.3)	19.7 (3.8)
100	124.4 (1.5)	131.8 (6.8)	16.2 (0.8)	26.1 (2.0)

* Flooding data were taken from an online archive with freely available measurements, which can be downloaded from http://live.waterbase.nl/waterbase_wns.cfm?taal=en

Table A2: The amount of subsamples per site for all the species collected.

Group	Species	0	10	25	35	45	55	100
Primary production								
Terrestrial plants	*Artemisia maritima*					5		5
	Atriplex portulacoides		5			5		5
	Elytrigia atherica							5
	Festuca rubra		5			5		5
	Limonium vulgare	5	5			5		
	Puccinellia maritima	5	5			5		
	Salicornia europaea	5	5					
	Soil organic matter		2			5		5
	Terrestrial organic matter		5			5		6
Marine prim. prod	Bacillariophyceae (Diatoms)	5	5			5		4
	Particulate organic matter		5			4		3
	Fucus vesiculosus	5	4			5		4
Herbivores	*Bledius* sp.	5	1					
	Cassida vittata	4	4	3	4			
	Auchenorrhyncha spp.							1
	Chrysomelidae sp.		1	1	1	3	1	1
	Elateridae sp.	1	1	1			2	
	Curculionidea sp.		2	1	3	2	4	
Carnivores	*Bembidion minimum*		3	2	3	5	2	5
	Clubiona stagnatilis	5	1	2	1	2	5	1
	Coccinella sedecumpunctata	5		3		2		1
	Dyscherius globusus		3	5	4	2		2
	Ichneumonoidea spp.	1	1	1				1
	Erigonidae spp.	4	5	3	5	2	3	3
	Pardosa pubeckensis	5	4	5	4	5	5	4
	Pogonus chalceus			2		1		
	Salda littoralis		1	4	4	3	3	1
	Tytthaspis sedecumpunctata		2		2	4	5	1
Detrivores	*Helophorus brevipalpis*							4
	Isotoma riparia		1				2	
	Platynothrus sp.			1				
	Symplecta stictica		2	4				
	Nemotelus sp.		3	2	1	4	3	
	Ochthebius marinus		4	4	3	3	1	
	Orchestia gammarellus	4	5	4	5	5	5	5
	Ovatella myosotis		4	5	5	5	5	5
	Phyllocia moscorum							1
	Fucellia maritima	4						
	Enchytraea sp.	5						

Every subsample is composed of at least 5 individuals. Not all species were identified up to the species level, those were given a 'sp.' behind the genus name. When more than one species was collected per taxa, 'spp.' is given behind the genus name.

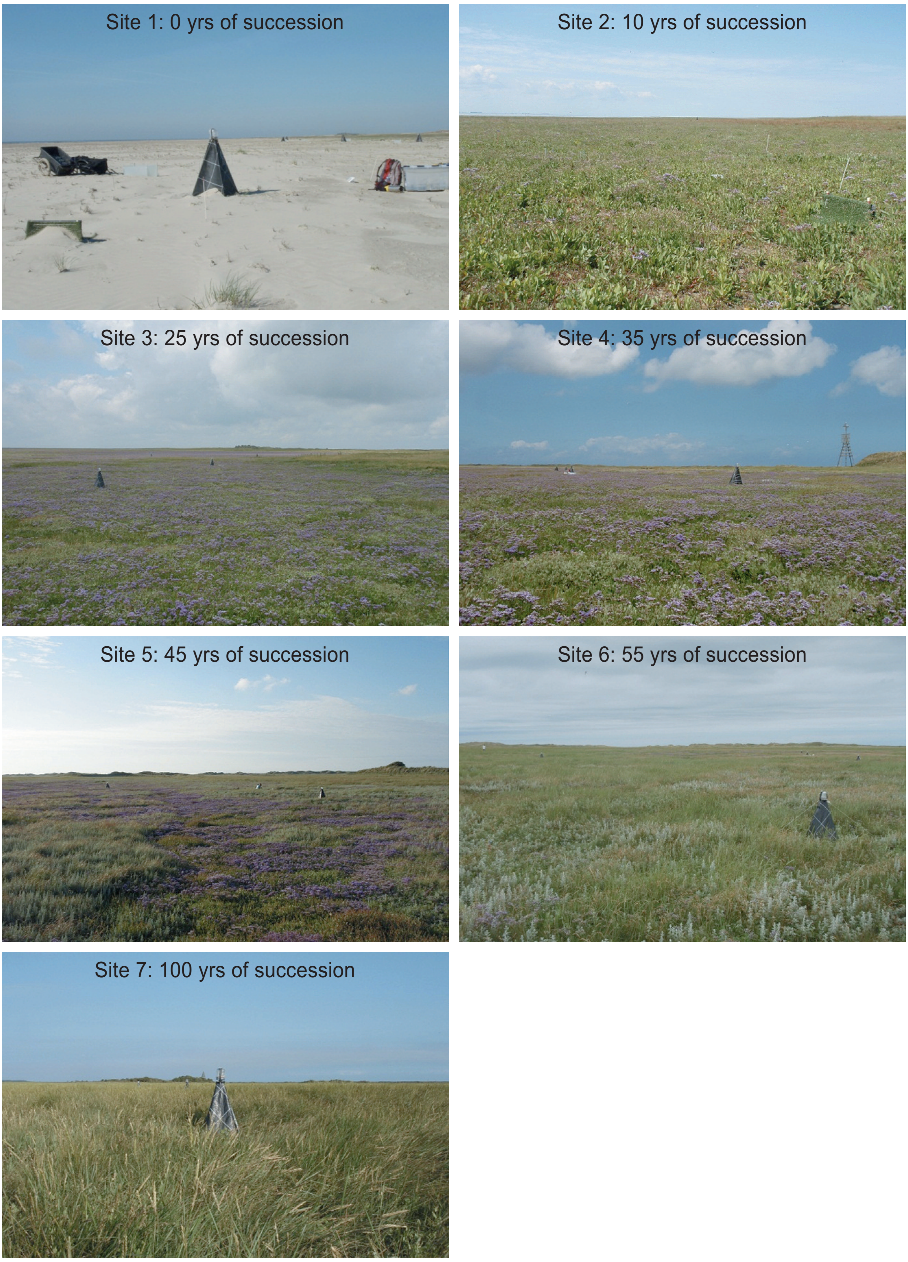
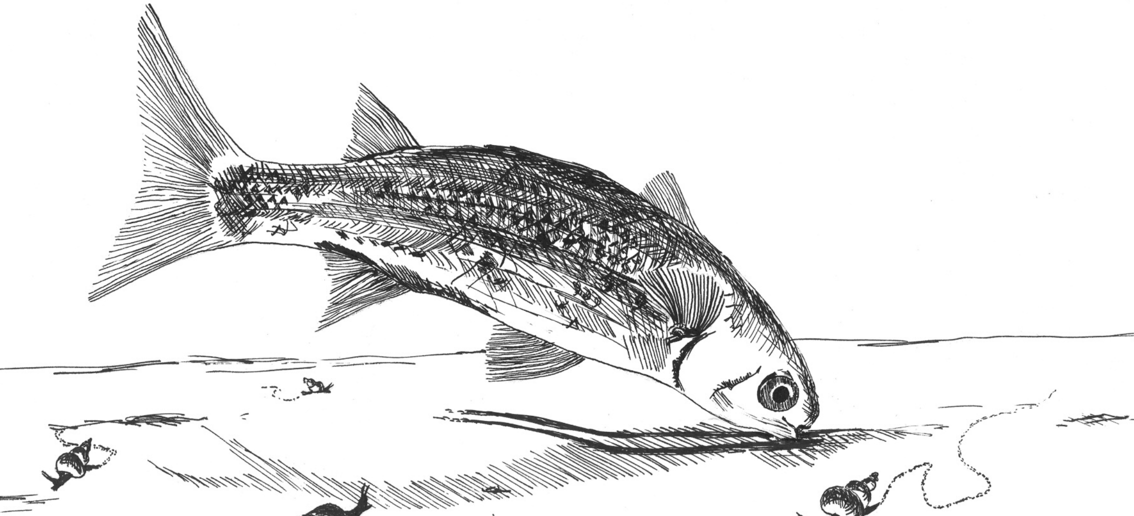


Figure A1: Photo's from each of the sites, July 2008.



CHAPTER 3

Benthic primary producers are key to sustain the Wadden Sea food web: stable carbon isotope analysis at landscape scale

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Abstract

Coastal food webs can be supported by local benthic or pelagic primary producers and by the import of organic matter. Distinguishing between these energy sources is essential for our understanding of ecosystem functioning. However, the relative contribution of these components to the food web at the landscape scale is often unclear, as many studies lack good taxonomic and spatial resolution across large areas. Here, using stable carbon isotopes, we report on the primary carbon sources for consumers and their spatial variability across one of the world's largest intertidal ecosystems (Dutch Wadden Sea; 1460 km² intertidal surface area), at an exceptionally high taxonomic (178 species) and spatial resolution (9165 samples from 839 locations). The absence of overlap in $\delta^{13}C$ values between consumers and terrestrial organic matter suggests that benthic and pelagic producers dominate carbon input into this food web. In combination with the consistent enrichment of benthic primary producers ($\delta^{13}C$ −16.3‰) relative to pelagic primary producers ($\delta^{13}C$ −18.8) across the landscape, this allowed the use of a two-food source isotope-mixing model. This spatially resolved modelling revealed that benthic primary producers (microphytobenthos) are the most important energy source for the majority of consumers at higher trophic levels (worms, molluscs, crustaceans, fish and birds), and thus to the whole food web. In addition, we found large spatial heterogeneity in the $\delta^{13}C$ values of benthic primary producers ($\delta^{13}C$ −19.2 to −11.5‰) and primary consumers ($\delta^{13}C$ −25.5 to −9.9‰), emphasizing the need for spatially explicit sampling of benthic and pelagic primary producers in coastal ecosystems. Our findings have important implications for our understanding of the functioning of ecological networks and for the management of coastal ecosystems.

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Introduction

The impact of environmental changes (e.g., eutrophication, hypoxia, ocean acidification) on species in ecosystems is often induced from the base of food webs (Paine 1980, Hoegh-Guldberg and Bruno 2010, Middelburg 2014). Food webs of estuarine ecosystems are fuelled by a variety of sources. Energy for heterotrophic organisms at higher trophic levels can originate as allochthonous organic matter from land via rivers, or from the sea via tidal currents. Alternatively, energy can be fixed internally by autochthonous pelagic or benthic primary production (Kamermans 1994, Herman et al. 2000) although the contribution of the latter is often undervalued in these systems (Tatara 1981, Barnes and Hughes 1999). In many food webs, the balance between the different energy sources for food webs is likely affected by coastal engineering, bottom disturbance (e.g. dredging, fishing), eutrophication and land use changes (Tewfik et al. 2005, Howe and Simenstad 2007). Yet, studies quantifying the main energy sources for diverse species of different trophic levels in estuarine food webs across geographic space are rare. Existing studies frequently focus on specific taxonomic groups such as molluscs and worms (Herman et al. 2000), on microbes and microfauna (Middelburg et al. 2000), on phytoplankton and macrozoobenthos, or focus on budget studies of carbon (energy) flow (Kuipers et al. 1981). In addition, despite the increasing evidence of high spatial heterogeneity in coastal ecosystems (Compton et al. 2013), spatial variation in carbon or food sources is generally rarely studied in estuarine intertidal ecosystems, as samples typically cover relatively small areas, and results are not reported in a spatially explicit manner (Herman et al. 2000, Middelburg et al. 2000, Catry et al. 2016)..

Stable carbon isotope measurements provide an important tool for unravelling the energy transfer and carbon sources in food webs (Middelburg 2014). Primary producers often differ in $\delta^{13}C$ values due to differences in carbon substrate (atmospheric carbon dioxide or dissolved inorganic carbon) and in carbon isotope fractionation during photosynthesis (Fry 2006). For example, CO_2 limitation during carbon fixation across the stagnant boundary layers of benthic algae explains their less negative $\delta^{13}C$ values compared to pelagic algae (France 1995). Carbon assimilated by higher consumers can be traced back to the basal resource, as the $\delta^{13}C$ of consumers largely reflects the $\delta^{13}C$ of primary producers at the base of the food web (De Niro and Epstein 1978, Fry 2006). As carbon isotopic signatures differ between marine benthic primary producers (Currin et al. 1995, Stribling and Cornwell 1997, Riera et al. 1999), marine pelagic primary producers (phytoplankton produced locally or imported; $\delta^{13}C$ −22 – −20‰; (Currin et al. 1995, Creach et al. 1997) and terrestrial, riverine and estuarine carbon sources (Middelburg and Herman 2007), they can be used to trace the relative importance of different energy sources for consumers (Herman et al. 2000).

In this study, we investigated what fuels the Wadden Sea food web. We assessed the relative importance of organic carbon derived from pelagic and benthic primary producers and from imported organic matter to animals in the Dutch Wadden Sea food web using stable carbon isotope signatures of species from different trophic levels at the landscape scale. Here we measured the $\delta^{13}C$ values at a high spatial and taxonomic resolution totalling 9165 analysed samples, from 178 species that were collected at 839 sampling locations spread across 1460 km² of intertidal flat. This study is also societally relevant because of the high natural value of the Wadden Sea ecosystem (Boere and Piersma 2012) that currently faces major impacts of human activities (Wolff 1983, Piersma et al. 2001b, Eriksson et al. 2010, Davidson 2014).

Methods

Study Area

The Wadden Sea is one of the world's largest intertidal ecosystems (Eisma 1976), bordered by twelve major sandy barrier islands that shelter the tidal area against waves generated by north-westerly and northerly winds (Zagwijn 1986). It spans from The Netherlands to Denmark with an overall surface area of approximately 8000 km². The Dutch Wadden Sea accounts for ~2500 km² of which 1460 km² consist of intertidal mudflats (de Jonge et al. 1993, Wolff 2000). In its present form, it is relatively young (± 8,000 years old).

The Wadden Sea is often described as an estuarine environment due to a distinct input of fresh water and sediment directly from the rivers Eems, Weser, Elbe, IJssel (through sluices from Lake IJsselmeer and Lake Lauwersmeer), and indirect fresh water input from the rivers Meuse and Rhine transported along the Dutch North Sea coast. However, unlike many other estuarine and delta systems, local river influence is nowadays only of minor importance relative to sediment supply from the adjacent coastal zone (Arends 1833, Van Straaten and Kunnen 1957). This is likely the result of the closure of the "Zuiderzee" estuary (3200 km², now "Lake IJsselmeer") in 1932 and "Lauwerszee" (91 km², now "Lake Lauwersmeer") in 1969, leaving the small river Ems as the only river with a still open connection to this estuarine ecosystem. In addition, the Wadden Sea has one of the world's most heavily modified coastlines (Wolff 1983, Piersma et al. 2001b, Eriksson et al. 2010, Davidson 2014), and borders one of the most intensively used shallow seas worldwide: the North Sea. Despite these impacts, the Wadden Sea offers important ecosystem services: it functions as a nutrient filter (Verwey 1952); supports high biodiversity and fisheries by providing key habitat to approximately 2,700 marine species, including charismatic seals and porpoises (Zijlstra 1972, Kuipers 1977, Strasser 2002, Compton et al. 2013); and it is a key foraging and resting area along the Atlantic flyway for migratory shorebirds (Kam et al. 2004, Blew et al. 2005).

Sampling

Species composition and biomass data were collected during a spatially comprehensive monitoring campaign (Synoptic Intertidal Benthic Survey, SIBES) between June and October of 2008 to 2012 (Bijleveld et al. 2012, Compton et al. 2013). This sampling program covers the entire intertidal of the Dutch Wadden Sea and consists of gridded samples taken at 500 m intervals and additional random samples (~4500 samples per year). As samples were collected from June to September, sampling was conducted haphazardly in geographic space over these six weeks to ensure that there was no temporal bias in the sampling and thus in our estimates. Depending on the tide, sampling locations were accessed either by foot or from a small boat. Sediment cores (25 cm depth, core surface of 0.018 m^2) were sieved on a 1 mm squared mesh sieve in the field, after which all organisms remaining on the sieve were stored for later identification, to species level or the finest taxonomic level possible, and for counting in the laboratory (NIOZ, Texel). In addition, biomass (ash free dry mass; AFDM) of each individual or of multiple individuals of the same species (for shells <8 mm) was determined. These samples were first dried for 2–3 days at 60°C and then incinerated for 5 h at 560°C. Biomass was then estimated by subtracting the dry from the ash weight (Bijleveld et al. 2012, Compton et al. 2013). Species were selected for stable isotope analysis when they accounted for more than 0.1% of the total average biomass, or when the species occurred (frequency of occurrence) in more than 10% of the sampled sites.

To select the most abundant benthic consumer species for the food source contribution (stable carbon isotope) analysis we calculated the average biomass (g AFDM m^{-2}) and the number of sites where a species was observed (%), and ranked species according to these two criteria (Table 3.1, Table S1). In total, 35 species were selected that together accounted for 99% of the total benthic biomass (Table 3.1).

$\delta^{13}C$ analysis

For the stable carbon isotope analysis, we randomly collected samples of benthos, macro-algae, seagrasses and higher consumers across geographic space, while the SIBES survey was conducted, between June and September of 2011 – 2014 (see Table S1 for an overview). Depending on the species type and size, in the laboratory we either used the muscle tissue (fish, crustaceans and bivalves), soft tissue (other invertebrates), blood plasma (birds), or whole organisms (smaller species or individuals) to estimate the isotope ratios. Fresh leaf material from macro-algae and seagrasses was also used as material for this analysis. All material was rinsed with demineralised water, freeze-dried for up to 96 h, ground and decalcified (by adding HCl) if required. For each species both acidified and non-acidified biomass were analyzed for $\delta^{13}C$ to determine whether acidification was required. When a significant depletion in $\delta^{13}C$ was observed between the acidified and non-acidified biomass for a species, its biomass was acidified for all smaller individuals of the whole data set. Homogenized samples (ca. 0.4 – 2 mg, depending on species) were weighed into tin cups, or when acidified in silver cups, and analysed for stable carbon isotope composition with a Flash 2000 elemental analyzer coupled online with a Delta V Advantage-isotope monitoring mass spectrometer (irmMS, Thermo Scientific). Stable carbon isotope ratios are expressed in the delta (δ) notation ($\delta^{13}C$) relative to Vienna PDB. Average reproducibility based on replicate measurements was ~0.18 ‰.

To establish benthic and pelagic baselines of $\delta^{13}C$ values we used proxies of long-lived primary consumers (see below) of which the diet is well known. This method is often used to indirectly characterize baseline resources because it integrates the variation in $\delta^{13}C$ over time (Cabana and Rasmussen 1996, Vander Zanden et al. 1999, Post 2002, Marty and Planas 2008, Middelburg 2014). By using proxies for benthic and pelagic primary producers, we avoided problems often encountered when establishing $\delta^{13}C$ baseline values based on direct measurements of small primary producers, for instance the physical separation of sources, labour-intensive methods (cell-specific or compound specific isotope measurements) and temporal variability caused by high turnover (Middelburg 2014).

As a proxy for pelagic producer $\delta^{13}C$ values, we used the $\delta^{13}C$ values of *Mytilus edulis* (blue mussel, an obligatory suspension feeder). *M. edulis* was collected from buoys set in deep channels where the input of resuspended material and terrestrial detritus was minimal. To validate this proxy, we sampled suspended particulate organic matter (POM). POM was collected by filtering 5 l of water (collected from gullies at neap tide) over pre-combusted Whatman GF/F glass fiber filters. Filters were dried for 48 h at 60°C before analysis. The $\delta^{13}C$ values of POM and the blue mussel were similar (average $\delta^{13}C$ −18.9 ±0.1 and −18.8 ±0.1‰, $P > 0.05$), supporting the use of blue mussels from buoys as a proxy for pelagic production.

As a proxy for the $\delta^{13}C$ values of benthic primary producers (also called microphytobenthos: the microscopic photosynthetic organisms living on the sediment surface that mainly consist of diatoms and cyanobacteria), we used the benthic algal consumer *Peringia ulvae* (mud snail or Laver spire shell, previously named *Hydrobia ulvae*) (López-Figueroa and Niell 1988). Although *P. ulvae* might not exclusively feed on microphytobenthos, prior work has shown that microphytobenthos is a primary food source (Herman et al. 2000). To validate this proxy we scraped benthic diatoms from the sediment surface at a selection of sites. After migration through a mesh (100 μm) into combusted sand, diatoms were collected in filtered seawater and filtered

Table 3.1 (right): Contribution of benthic primary production (%) to the diet of the 35 most common benthos species of the intertidal flats of the Dutch Wadden Sea. Presented is the average biomass (mean AFDM g/m^2), and the relative percentages of sites (%) were species were observed for 35 species that together constituted 99.3% of the total biomass across sampling sites in 2008–2012 (nr. sites 3465–4375). The rank of each species according to biomass and species prevalence is shown in superscript. Average δ^{13}C values (± se) and their range are presented. Note that the use of an average δ^{13}C value of a species does not translate directly into the amount of contribution by benthic food sources for some species. * *M. edulis* samples here represent mussels sampled from intertidal flats that rely on resuspended benthic primary producers, and δ^{13}C values therefore differ from of "pelagic" mussels sampled from buoys, high up in the water column. ** *P. ulvae* values are used as a proxy for contribution of benthic food sources, no contributions were calculated for proxies. We chose to constrain values between 5 and 95% due to the high spatial variability and average extrapolated values.

over a Whatman GF/F glass fibre filter (Eaton and Moss 1966) and analyzed for δ^{13}C. The *P. ulvae* and microphytobenthos δ^{13}C values showed large overlap in their frequency distributions, with *P. ulvae* having a narrower range (−19.2 to −11.5 ‰; average −16.3 ±0.1‰) than microphytobenthos (−21.7 to −10.7 ‰, with average of −15.7 ±0.2‰) (Figure S1C), supporting the use of *P. ulvae* as a proxy for benthic production.

Geographical mapping of δ^{13}C values

δ^{13}C values of benthic primary producers, pelagic primary producers, and three representative consumers were spatially interpolated over the Dutch Wadden Sea using the ordinary Kriging function in ArcGIS (version 10.3) based on a spherical semivariogram model. The kriging - output cell size matched the sampling grid (500 m, SIBES), and a δ^{13}C value for a cell was obtained using the values of the six closest sampling points for that organism with a maximum range of 5 km. All interpolated maps, except the map of pelagic primary production, were clipped to intertidal areas and to areas with a maximum distance of 5 km to the nearest sampling location. Sampling locations used for interpolating δ^{13}C values of primary producers amounted to 31 locations (111 samples) for pelagic primary producers and 102 locations (135 samples) for benthic primary producers. To illustrate spatial heterogeneity in primary consumers, three consumers (common cockle *Cerastoderma edule*; ragworm *Hediste diversicolor*, formerly known as *Nereis diversicolor*; and Baltic tellin *Limecola balthica* formerly known as *Macoma balthica*) were selected because of their high biomass (mean of 5.8, 0.9 and 0.9 g AFDM m^{-2} across years) and high frequency of occurrence (21, 31, 31 %) , their prominent ecological role (Degraer et al. 2008) and their different feeding strategies, i.e. suspension feeder, scavenger, and facultative deposit feeder respectively.

		Benthic contribution	Biomass		Obs. of species		$\delta^{13}C$		
Group	Species	av % (±se)	g m^{-2}	rank	% sites	rank	av (±se)	min	max
Molluscs	*Cerastoderma edule*	<5 (5)	5.8	1	21	10	−18.6 (1.3)	−21.9	−6.5
	Mya arenaria	63 (21)	2.7	2	10	17	−17.2 (3.5)	−21.1	−3.4
	Ensis directus	23 (12)	1.7	4	11	16	−18.2 (1.5)	−21.0	−11.8
	Macoma balthica	>95 (13)	0.9	8	31	5	−16.0 (1.9)	−20.9	−7.9
	Crassostrea gigas	19 (15)	0.9	9	0.4	53	−17.8 (0.9)	−20.8	−16.8
	*Mytilus edulis**		0.8	10	2	35	−18.8 (0.1)	−23.5	−9.0
	*Peringia ulvae***		0.5	11	11	14	−16.3 (0.1)	−25.5	−8.4
	Scrobicularia plana	>95 (46)	0.3	13	4	28	−15.7 (1.8)	−19.7	−11.1
	Littorina littorea	>95 (25)	0.1	20	0.5	52	−14.2 (1.4)	−17.1	−10.6
	Petricola pholadiformis	58 (54)	0.02	26	0.3	57	−18.1 (0.6)	−19.0	−17.2
	Tellina tenuis	55 (30)	0.02	27	1	44	−16.5 (1.0)	−16.5	−16.5
	Abra tenuis	>95 (30)	0.02	30	2	36	−13.2 (2.5)	−16.5	−6.4
Annelids	*Arenicola marina*	62 (15)	2.4	3	28	7	−16.3 (1.2)	−20.7	−13.3
	Lanice conchilega	<5 (28)	1.1	5	19	11	−17.9 (1.2)	−20.3	−14.9
	Scoloplos armiger	84 (7)	0.9	6	58	1	−16.4 (1.3)	−19.5	−13.2
	Hediste diversicolor	>95 (10)	0.9	7	31	6	−16.1 (1.7)	−20.2	−9.9
	Marenzelleria viridis	<5 (19)	0.3	12	32	4	−18.1 (1.0)	−19.5	−16.0
	Alitta virens	95 (41)	0.2	15	2	39	−17.6 (0.2)	−17.7	−17.5
	Nephtys hombergii	>95 (52)	0.2	17	13	13	−15.0 (1.0)	−17.9	−13.8
	Alitta succinea	>95 (128)	0.1	18	8	21	−17.2 (1.5)	−19.2	−12.7
	Capitella capitata	<5 (18)	0.1	19	38	2	−17.9 (0.5)	−18.5	−17.2
	Heteromastus filiformis	<5 (8)	0.1	21	22	9	−17.7 (1.3)	−19.7	−14.2
	Eunereis longissima	63 (45)	0.1	23	2	40	−16.8 (1.9)	−18.4	−13.5
	Eteone longa	>95 (11)	0.05	24	36	3	−15.6 (1.2)	−17.8	−12.6
	Pygospio elegans	62 (35)	0.02	28	10	18	−16.5 (1.9)	−18.6	−13.5
	Aphelochaeta marioni	27 (12)	0.02	29	9	20	−17.7 (0.7)	−18.2	−16.6
	Bylgides sarsi	>95 (217)	0.01	32	6	23	−17.1 (1.0)	−18.5	−15.4
	Phyllodoce mucosa	72 (17)	0.01	33	11	15	−18.2 (1.0)	−18.2	−18.2
	Polydora cornuta	78 (27)	0.01	34	9	19	−18.8 (1.0)	−18.8	−18.8
	Oligochaeta sp.	74 (42)	0.01	35	5	26	−14.7 (1.8)	−18.7	−13.4
Crustaceans	*Carcinus maenas*	>95 (10)	0.3	14	6	22	−15.8 (1.2)	−23.0	−11.3
	Corophium sp.	<5 (19)	0.2	16	18	12	−18.0 (2.3)	−21.5	−12.9
	Urothoe sp.	>95 (17)	0.1	22	25	8	−15.9 (1.4)	−19.0	−11.3
	Crangon crangon	>95 (18)	0.02	25	5	25	−15.0 (1.7)	−23.2	−11.4
	Bathyporeia sp.	>95 (101)	0.01	31	5	24	−14.8 (2.7)	−16.6	−12.9

* proxy for pelagic producers,
** proxy for benthic producers

Food source estimation

The relative contribution of benthic and pelagic food sources was estimated for each consumer at each sampled location to account for the spatial variability in $\delta^{13}C$ of the two food sources. For each coordinate where a consumer was sampled, $\delta^{13}C$ values were extracted from extrapolated benthic and pelagic primary producer maps using the function "add values to points" in ArcGIS (Figure 3.3). As the $\delta^{13}C$ of organisms reflects the $\delta^{13}C$ of primary producers at the base of the food web, stable isotope mixing equations can be used to infer the carbon (energy) supply to consumers (Phillips and Gregg 2003, Tewfik et al. 2005). Here, a simple two-end member isotope-mixing model was used to calculate the contribution of benthic primary producer carbon ($f_{\text{benthic primary producers}}$) to each consumer at each sampled location:

$$f_{\text{benthic primary producers}}\ (\%) = (\delta^{13}C_{\text{consumer}} - \delta^{13}C_{\text{pelagic primary producer}}) / (\delta^{13}C_{\text{benthic primary producers}} - \delta^{13}C_{\text{pelagic primary producer}}) \times 100$$

where $\delta^{13}C_{\text{pelagic primary producer}}$ and $\delta^{13}C_{\text{benthic primary producer}}$ are the carbon values of the proxies for primary producers (buoy-attached blue mussel and mud snail, respectively). The resulting values were averaged over all sampling locations, yielding the average contribution of benthic production for all measured members of the Wadden Sea food web (Figure 3.1). The rationale for using this two-end member mixing model is presented later in the results section.

Our simple two-end member approach neglects trophic fractionation and therefore might overestimate the contribution of the ^{13}C-rich food sources, i.e. benthic primary producers, with about 10–20 % (see discussion). The resulting $f_{\text{benthic primary producers}}$ (%) was spatially extrapolated using the method described above and presented in maps (Figure 3.2 and 3.3). To obtain the most robust estimation of the relative contribution of the two sources in space and to account for the high spatial variability we chose to constrain values of the food source contribution between 5% and 95% confidence intervals.

Results

Benthic species biomass and density

The selection procedure for the most abundant benthic consumers (see Methods) yielded 35 out of a total of 111 benthic species, which together accounted for 99.3% of the total benthic intertidal biomass. The mean benthos biomass across sampling points was on average 25 g AFDM m^{-2} per year over the sampling period 2008–2012 (n = 3467 – 4179). Species ranking, average biomass and the frequency of occurrence

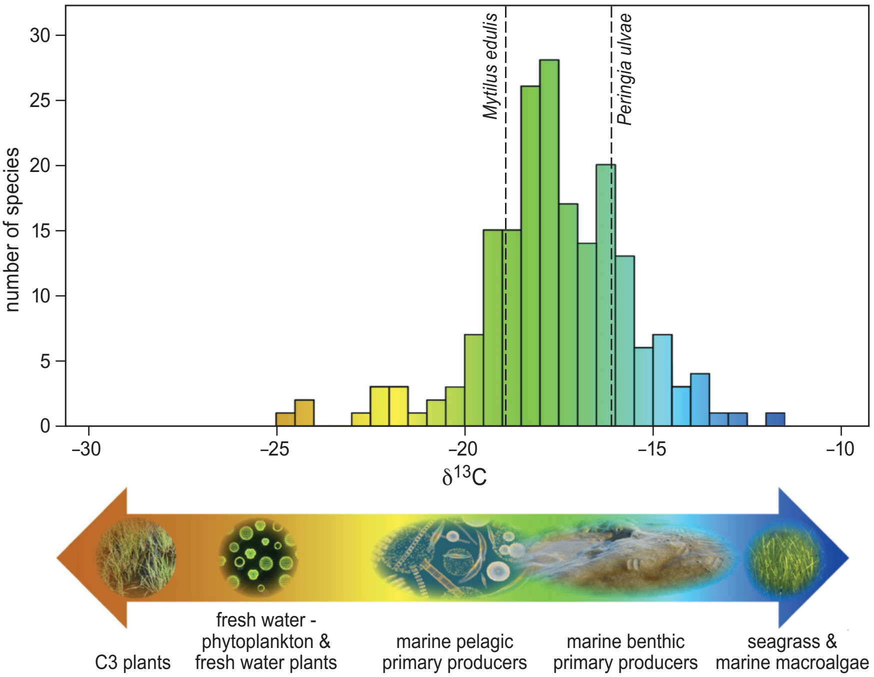

Figure 3.1: Frequency distribution of median δ^{13}C values of most species of the Dutch Wadden Sea (178 species, 9165 samples) shows that energy for the Wadden Sea ecosystem is provided by locally produced organic matter (benthic primary producers, δ^{13}C range: −19.2 – −11.5 ‰) and to a lesser extent by pelagic producers (δ^{13}C range: −23.3 – −17.4 ‰) and there are no indications for significant external (terrestrial) inputs of organic matter. This graph provides a rationale behind our simple two food source mixing model that includes only benthic and pelagic primary producers as a source. Dashed lines show median δ^{13}C values of the proxies used to map benthic (*Peringia ulvae*) and pelagic (*Mytilus edulis*) producers.

are presented in Table 3.1. The three benthic primary consumers contributing the highest mean biomass across the Dutch Wadden Sea were the common cockle (*Cerastoderma edule*; 5.8 g AFDM m^{-2}), the soft-shell clam (*Mya arenaria*; 2.7 g AFDM m^{-2}), and the lugworm (*A. marina*: 2.4 g AFDM m^{-2}).

Validation of two-food source mixing model

The frequency distribution of median δ^{13}C values of 178 species (Figure 3.1) showed that values varied from −25 to −11.5 ‰ with 95% of the values falling between −20 and −14.5 ‰. Thus, the δ^{13}C values of almost all consumers in the Wadden Sea food web fall within the range of the proxies for benthic primary producers (mud snail;

δ^{13}C range −19.2 to −11.5 ‰; average −16.3 ±0.1‰) and for pelagic phytoplankton living in the Wadden Sea or imported by tides from the North Sea (buoy-attached blue mussel; (δ^{13}C range −23.5 to −17.3 ‰; average ±SE −18.8 ±0.1‰). To test for temporal variability, relations between δ^{13}C and time were analysed for some highly dominant species and found to be not significant (e.g. *P. ulvae*; R= 0.0025). Naturally, this simplification does not exclude contributions of other food sources on a local scale in this highly dynamic system

Spatial heterogeneity of δ^{13}C values: primary producers

The spatial patterns of δ^{13}C values of our proxies for benthic (*P. ulvae*) and pelagic primary producers (*M. edulis*) were clearly different (Figure 3.2) and showed little overlap (Figure S1). Isotope data of *M. edulis* (blue mussels collected from buoys) indicated that their δ^{13}C values were geographically uniform across the Wadden Sea (averaging −18.8 ±0.12‰). In contrast, benthic primary producers showed a more heterogeneous pattern in space with significantly less negative values of δ^{13}C on average (−16.3 ±0.12‰) than the primary producers. The difference between δ^{13}C values of benthic and pelagic primary producers averaged 2.15 ±0.11‰ (range 0 – 4.9 ‰). Although the average δ^{13}C ranges of both primary producers overlapped (Figure 3.2), at a landscape scale this overlap in δ^{13}C values was absent due to spatial heterogeneity. This suggests that, although the difference in isotopic composition was relatively small, we could distinguish benthic and pelagic energy sources in consumers.

Spatial heterogeneity of δ^{13}C values: consumers

The stable carbon isotope values of benthic consumers showed high spatial heterogeneity (e.g. Figure S2) for species that either foraged on benthic (subfigures A and B) or pelagic resources (subfigure C) and these patterns varied between different consumers. To illustrate the different types of spatial patterns in δ^{13}C values, we constructed maps of three abundant benthic primary consumers that represent species with 3 different feeding strategies (Figure 3.3). Spatial pattern in the δ^{13}C values of the ragworm (*Hediste diversicolor*), a scavenger, was heterogeneous (Figure 3.3A). Stable carbon isotope values of the common cockle, a suspension feeder, reflected the dominance of pelagic primary production over a large part of the Wadden Sea. Only in a small restricted area (high intertidal muddy areas south of Terschelling Island) δ^{13}C values reflected dominance of benthic producers (>50% benthic contribution) (Figure 3.3C). The δ^{13}C patterns of the Baltic tellin (*Limecola balthica*, Figure 3.3B), a facultative deposit feeder, showed high spatial heterogeneity mainly with values indicating a high benthic contribution to its diet, but in some areas values indicated a high pelagic contribution to the diet as well.

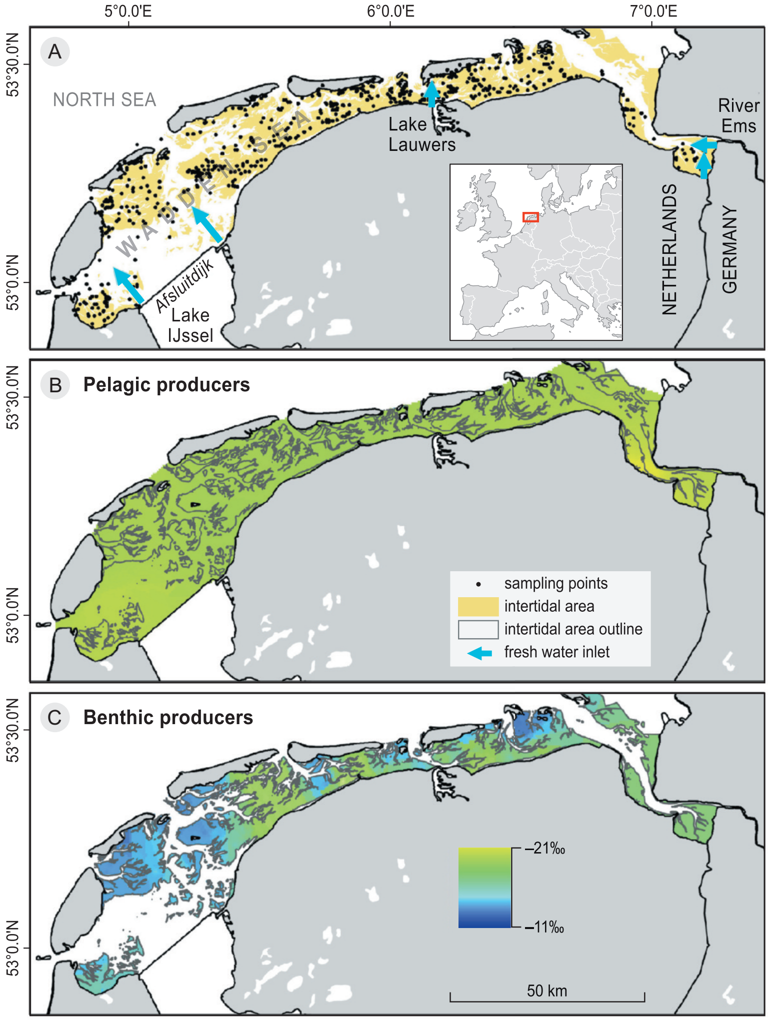

Figure 3.2: Map of the Dutch Wadden Sea with (A) sampling locations (n = 839) for carbon isotope analysis (black dots, 9165 samples), fresh water inlets (blue arrows) and intertidal areas (orange, ±1460 km^2), (B) extrapolated $\delta^{13}C$ stable isotope values of pelagic primary producers – using pelagic first consumers (*Mytilus edulis* from buoys) as a proxy, and (C) extrapolated $\delta^{13}C$ stable isotope values of benthic primary producers using benthic first consumer (*Peringia ulvae*) as a proxy. Pelagic primary producers show a rather uniform pattern with relatively negative $\delta^{13}C$ values ($\delta^{13}C$ min: −23.3, max: ±−17.4‰). Benthic primary producers show a more heterogeneous pattern with less negative $\delta^{13}C$ values ($\delta^{13}C$ min ±−19.2, max ±−11.5‰). Note that the extent of the geographical mapping of primary producers was adjusted to their habitat; Benthic primary producer $\delta^{13}C$ values were geographically mapped to the intertidal area and pelagic primary producer $\delta^{13}C$ values were extrapolated over the whole Dutch Wadden Sea.

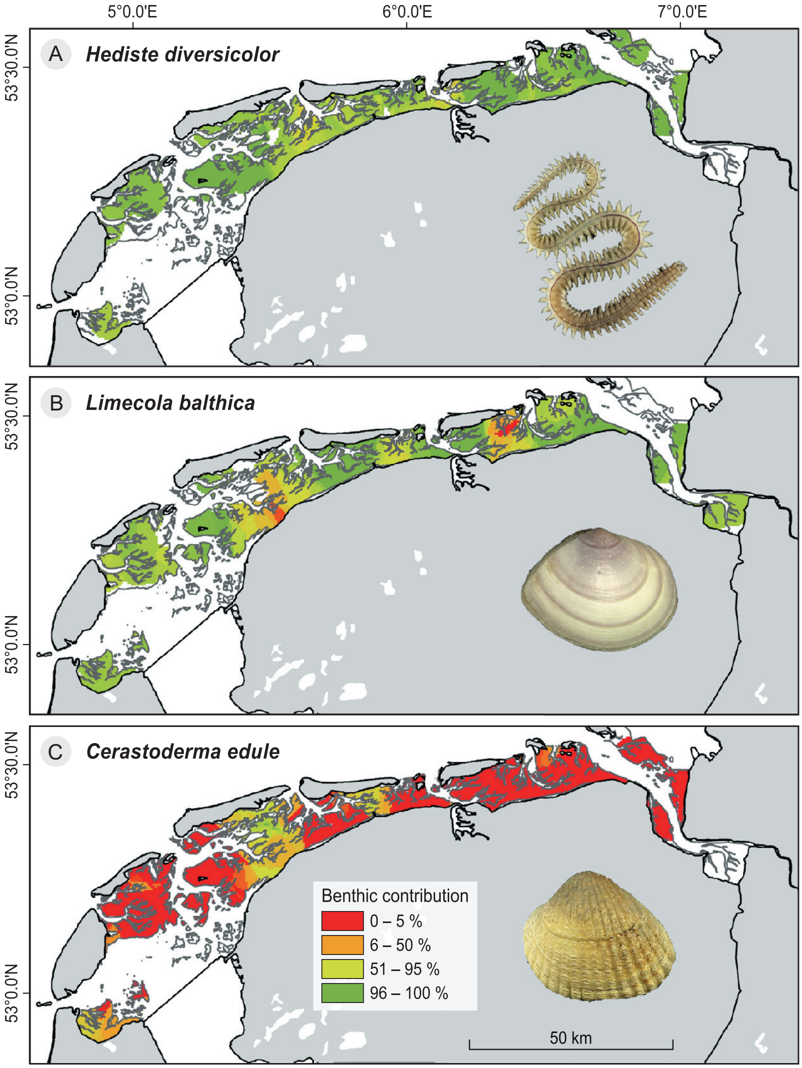

Figure 3.3: Relative contribution of benthic primary production for key consumers; (A) *Hediste diversicolor* (n = 120), (B) *Limecola balthica* (n = 139), (C) *Cerastoderma edule* (n = 346) extrapolated over the Dutch Wadden Sea. Green: energy predominantly from benthic primary production, Red: energy predominantly from pelagic primary production.

Estimation of food sources

The results from our spatially resolved, two-food sources mixing model showed that benthic primary producers were the dominant food source for 74% of the 35 most abundant benthic species (Figure 3.4). Species that depended predominantly on benthic primary production together accounted for 52% of total benthic biomass (see discussion for an explanation on this conservative estimation).

Our spatially resolved, two-food sources mixing model was also used to calculate the $\delta^{13}C$ food source contribution for 143 other, less abundant, species (Table S1). Benthic primary producers were also important for these less abundant species, and their contribution dominated in 42 species. These 42 species were typically more abundant in benthic samples (e.g. see higher "n" values in Table S1) compared to species that depended more on pelagic primary production. However, the contribution of the two food sources to the total community carbon flow could not be quantified as biomass data was unavailable for many species of higher trophic levels (e.g. fish).

Discussion

Thorough quantifications of the main food sources for heterotrophic species at different trophic levels in coastal food webs are rare, but are needed to understand the functioning of food webs. Tidal systems such as the Wadden Sea are home to many benthic and pelagic primary producers and also receive organic matter from adjacent systems such as the North Sea and rivers (Kuipers et al. 1981, van Raaphorst and van der Veer 1990, Bouillon et al. 2011). With our two-food source mixing model, based on the pelagic and benthic primary producer proxies, the food contribution was resolved for 91% of the benthic species. Some of the non-resolved species were migrants (e.g. European river lamprey, *Lampetra fluviatilis* and Brent goose, *Branta bernicla*). Others were worms that showed predominant utilization of pelagic carbon sources but actually live buried deep in the mud, out of reach of pelagic sources, and are most likely to feed on bacteria (e.g. Spionid polychaete, *Marenzelleria viridis*; Gallery worm, *Capitella capitata*; Red thread worm, *Heteromastus filiformis*).

Our results showed that benthic primary producers that thrive on the intertidal mudflats (primarily microphytobenthos) supported the majority of consumers in the Wadden Sea food web (Figure 3.5). Phytoplanktonic organic matter (POM) had $\delta^{13}C$ values higher than −22‰ (range $\delta^{13}C$ −22.5 – −15.7 ‰), typical for POM (Middelburg and Herman 2007), but distinctly different from terrestrial, riverine and estuarine carbon resources (with $\delta^{13}C$ values typically in the range from −26‰ to −30 ‰; (Middelburg and Herman 2007). This suggests relatively low contributions of terres-

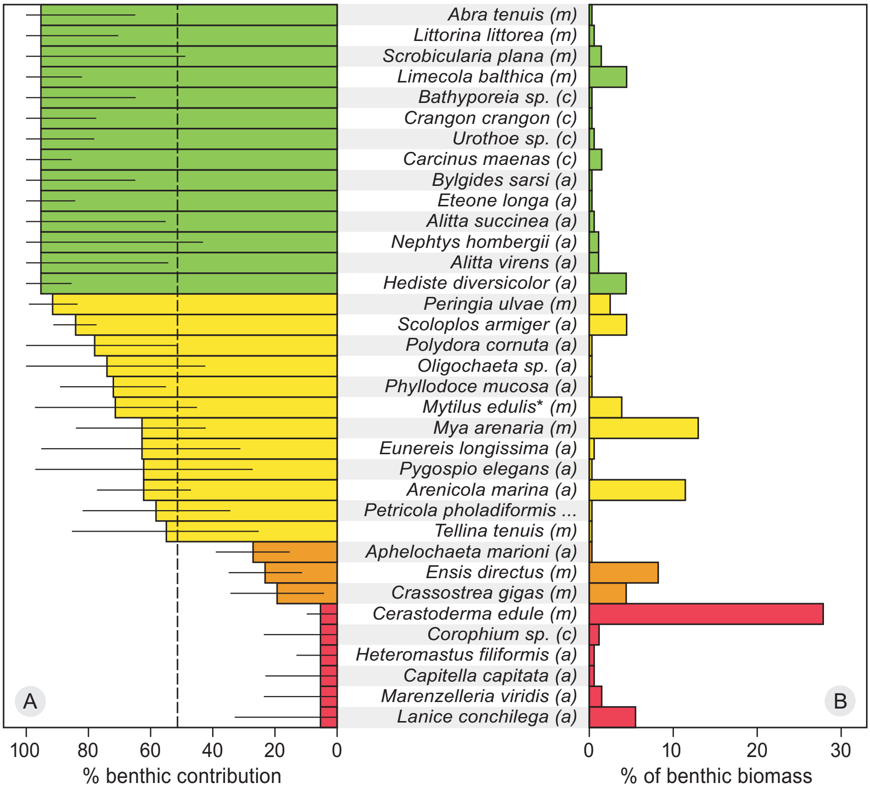

Figure 3.4: (A) Contribution of benthic primary production (%) to the 35 most common benthos species of the intertidal flats of the Dutch Wadden Sea, resulting from the two-food source mixing model. Presented are values (±SE) of contribution by benthic food sources averaged across sampling locations in the Dutch Wadden Sea for each species. In brackets the species' taxonomic class is presented; *m*: molluscs, *c*: crustacean, *a*: annelida. **M. edulis* here represent benthic mussels sampled on intertidal flats that rely on resuspended benthic primary producers, and $\delta^{13}C$ values therefore differ from the "pelagic" mussels sampled as from buoys as proxies for pelagic producers, high up in the water column, and (B) relative benthic biomass of 35 benthos species. Species that are dependent predominantly (>50%) on benthic primary contribution together account for 52% of total benthic biomass. The percentage of energy from benthic primary production is presented in colors: Green: >95%, yellow: 50–95 %, orange: 5–50 %, red: <5%. *note that this value an underestimation due to the fact that the common cockle that represents 23% of the biomass dependent on pelagic contribution, while in fact only 1% is harvestable (e.g. by shore birds such as red knot that only eat shells <12 mm) and available for higher organisms.

trial organic matter, which is therefore unlikely to be a major resource in the Wadden Sea at present. Another potential food source included macrophytes e.g. seagrasses ($\delta^{13}C$ values ranging between −15.2 and −10.9‰, average −13.0 ±0.2‰). Although these values potentially overlapped with some of the consumers they are unlikely to contribute significantly to the carbon flow and thus to consumers in the Dutch Wadden Sea ,as seagrasses are nearly ecologically extinct in the Dutch Wadden Sea (Folmer et al. 2016) and extensive macroalgae fields are lacking.

Our findings are fully consistent with the results of Herman et al. (2000) and Middelburg et al. (2000). These small-scale studies combined a natural abundance stable isotope approach with an isotope tracer study in the Wester Scheldt estuary and showed that benthic consumers in intertidal ecosystems depend heavily on benthic primary production. However, our results appear inconsistent with traditional diet studies in the Wadden Sea area that show that benthic consumers in intertidal ecosystems are primarily dependent on imported organic matter or local primary production in the water column. For example, in the Balgzand area of the Wadden Sea, the stomach contents of intertidal deposit and filter feeders (*Cerastoderma edule*, *Mya arenaria* and *Mytilus edulis*) suggested a dependence on pelagic algae (Kamermans 1994). Furthermore, studies in the same area suggested that phytoplankton production was the most important component to the organic matter budget (Colijn and de Jonge 1984). At this point, we cannot distinguish whether this discrepancy with previous literature is explained by (1) changes in ecosystem functioning between the 80's and the present (Philippart et al. 2000, Eriksson et al. 2010, van der Veer et al. 2015), (2) the methods used, or (3) the much more spatially restricted location of the Balgzand studies (Beukema et al. 2002), close to a freshwater outlet – a hypothesis that is supported by the more negative $\delta^{13}C$ POM values that we found here.

In terms of total available biomass, consumers depending on benthic and pelagic primary producers are similarly dominant in this system (Figure 3.4, Figure 3.5, Table S1). However, in terms of harvestable biomass (i.e. the biomass available for species higher up the food chain) benthic primary production is the most dominant food source. Although the common cockle is a pelagic consumer that is important for higher consumers and has a very high biomass (23%, relative to the total, based on 5-year monitoring data), its harvestable fraction for consumers can be low. For example, its harvestable fraction for birds like the red knot (*Calidris canutus*) only equates to 1% of the total available biomass, as it depends on the size of the bill (Zwarts et al. 1992, Bijleveld et al. 2015). Examples of consumers that depend on benthic production and might also exhibit size selectivity include *Limosa lapponica* (Duijns et al. 2013), and *Pluvialis squatarola* (Kersten and Piersma 1984) and Piersman T 1984), *Calidris alpina* (feeding on *Scoloplos armiger* pers. obs. T. Piersma). Correcting biomass contributions for other species would likely further increase the proportion of benthic carbon contribution. On the one hand, our estimation of the contribution of

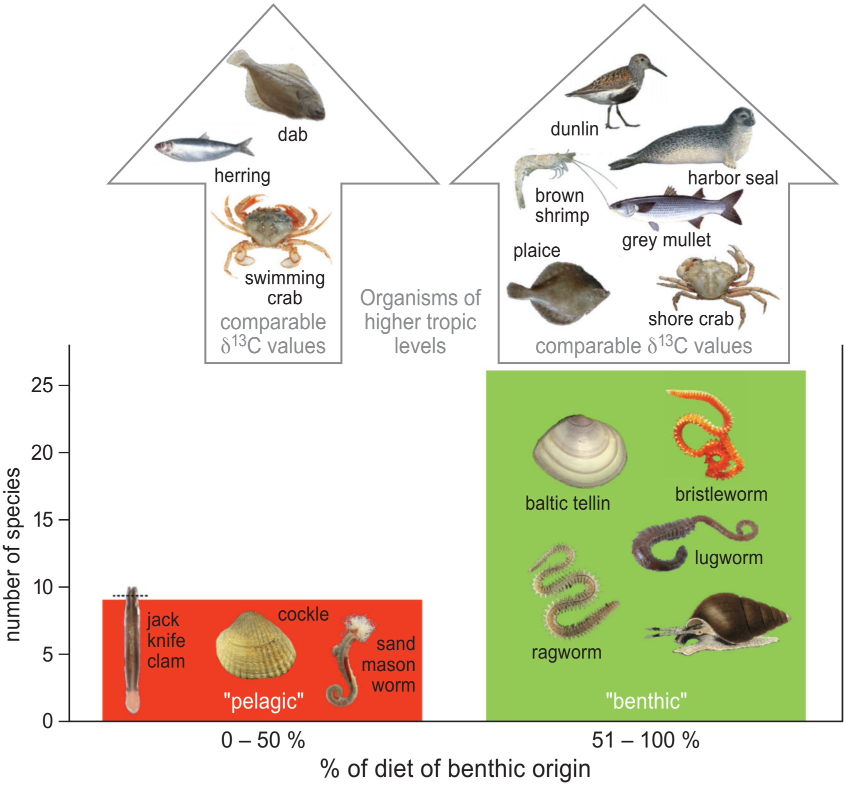

Figure 3.5: Conceptual diagram on the importance of benthic production to species higher up the food chain. Relative contribution of benthic primary production (%) to the diet of 35 most common benthos species of the intertidal flats of the Dutch Wadden Sea classified into 0–50 % (red) and 51–100 % dependence on benthic algae production (green). Bars include figures of some of the most abundant species of each 2 classes. Examples of species of higher trophic levels with "benthic" or "pelagic" of "intermediate" $\delta^{13}C$ signals are presented inside arrows (see appendix Table S1 for details).

benthic food sources in our study may be conservative because a small contribution of terrestrial, riverine and estuarine carbon resources to food resources (with more negative $\delta^{13}C$ values like in the Ems estuary) would increase the percentage of pelagic contribution in our two-food source mixing model and underestimate the contribution of benthic sources (with more positive $\delta^{13}C$ values compared to pelagic sources). On the other hand, the contribution of benthic food source may be slightly overestimated (10-20%) for higher trophic levels if carbon is enriched per trophic transfer.

Dominance of benthic food sources in coastal systems - Is it a common phenomenon?

To explore if the dominance of benthic food sources is a general phenomenon in coastal ecosystems worldwide, we compared $\delta^{13}C$ of producers and consumers in other coastal systems from data in the literature. In the Westerschelde (The Netherlands), 95% of the benthic macrobenthos (Herman et al. 2000), as well as bacteria and meiobenthos (Middelburg et al. 2000) were found to depend on benthic primary production. In the Seto inland Sea (Japan), 92% of demersal fish species showed $\delta^{13}C$ values between −17 to −13 ‰, likely indicating a high contribution of benthic primary producers to consumers at the top of food webs (Takai et al. 2002). This is further supported by a review on saltmarsh food webs throughout the East and Gulf coast of North America that found average $\delta^{13}C$ values between −16.3 and −13.9 ‰ for macrofauna species, similar to an average $\delta^{13}C$ value for benthic primary producers of −15.5‰ (Currin et al. 1995). Benthic primary production was also found to be dominant in salt marsh consumers in Mont St. Michel, France (Creach et al. 1997), saltmarsh mudflat infauna at Plum Island Estuary, USA (Galvan et al. 2008) and the Pearl River estuary, China (Lee 2000), for cockles in Marennes-Oleron Bay, France (Kang et al. 1999) and for prawns in Klang river creeks, Malaysia (Newell et al. 1995). Even in deeper coastal areas, the carbon subsidy of benthic food sources might still be high, as studies in the South Atlantic Bight (USA) found that microphytobenthos contributed 40% to the system's primary production at depths between 14–40 m (Jahnke et al. 2000). Although most of these studies only focused on a few species, typically covered small areas, and had a limited sample size, they seem to suggest that a dominance of benthic food sources is the rule rather than the exception in the deltas of this world.

Together the dominance of consumers' $\delta^{13}C$ values between those of the mud snail and buoy-attached blue mussel, and the lack of substantial terrestrial, riverine, macrophyte carbon resources suggested that a simple two-food source mixing model could be applied to determine the major sources of food for animals higher up the food web. Our assumption is supported by isotope studies on other intertidal areas that have also found that the main higher trophic levels fed highly selectively and relied primarily on microphytobenthos and pelagic primary production (van Oevelen et al. 2006). Naturally, this simplification does not exclude contributions of other food sources on a local scale in this highly dynamic system. The absolute distinction between pelagic and benthic algae in shallow, tidal systems is not straightforward because phytoplankton represents a dynamic mixture of benthic and pelagic algae due to intensive tidally driven resuspension-deposition cycles (Herman et al. 1999, Lucas et al. 2001). Stable isotope analysis provides information on the locus of carbon fixation (pelagic vs. benthic) and thus on the eventual energy source and not so much on where the carbon has been consumed. For instance, resuspended benthic algae consumed by suspension feeders will be recorded as a benthic contribution by stable isotope analysis. Stable isotope analysis integrates the assimilated diet across time and unlike stomach analysis is able to bypass the source determination of mixed particles in degraded forms (De Niro and Epstein 1978, Fry 2006).

Spatial patterning in carbon isotope values: Implications for understanding of marine food webs

Our high-resolution study enabled a detailed evaluation of spatial heterogeneity in the isotopic composition of food sources of benthic consumers in a coastal region and is among the most extensive stable isotope food web studies ever attempted. We found a remarkable degree of spatial heterogeneity, of especially benthic primary producer carbon isotope values, throughout our study area at scales larger than the patchy occurrence of the individual benthic species (Kraan et al. 2009). The cause for this large spatial variability is unclear. We found a positive correlation between benthic primary producer $\delta^{13}C$ values and the exposure time (hours without inundation) of each sampled location ($R^2 = 0.55$, Figure S3; i.e. less negative values with increased exposure to air) as measured from tidal elevation. This indicates that benthic producers on longer exposed areas have different $\delta^{13}C$ values than those in areas that are more frequently flooded or permanently submerged. Possibly different diatom species could dominate in different depth zones, and also explain some of the spatial variability (Henley et al. 2012). As thicker stagnant boundary layers around benthic algae increase diffusion limitation of CO_2 and consequently decrease overall fractionation (France 1995, Hopkinson et al. 2011), this could result in more positive $\delta^{13}C$ values in areas of lower flow velocities. Reduced water depth might also yield more positive $\delta^{13}C$ values, as benthic algae living higher on intertidal mudflats are more productive which generally results in decreased isotopic fractionation (Laws et al. 1995). Regardless of the exact cause, it is clear that benthic primary producers show an isotopic variability that is strongly influenced by geophysical, and therefore spatial factors.

Spatial patterning in carbon isotope values: Implications for food web sampling and modelling

Our study has important implications for future food web studies. Spatial heterogeneity in $\delta^{13}C$ values of primary producers has been reported for seagrasses (Fourqurean et al. 1997), phytoplankton (Boschker et al. 2005, Tamelander et al. 2009), coastal kelp (Simenstad et al. 1993) and salt marshes (Deegan and Garritt 1997), but has yet to be studied for benthic microalgae in marine environments. Our data showed that stable carbon isotopes of benthic primary producers are location-dependent. Consequently, modelling of reliable future food web studies should be adjusted and incorporate high-resolution spatial sampling of benthic primary producers, and not use extrapolations based on a limited number and/or local measurements. The relatively homogeneous stable carbon isotope pattern of cockles (Figure 3.2B), as determined here for the Wadden Sea, indicates that isotope food-web modelling for pelagic producers may perhaps be done using one single end-member that is independent of location. However, as this outcome may be specific for the Wadden Sea, we also recommend a high-resolution spatial sampling scheme for pelagic primary producers using proxies that are simple to collect. Specifically, environmental monitoring programs (Parr et al. 2003) need to include spatially explicit sampling of benthic and pelagic primary producers in coastal systems to improve our current understanding of food web functioning.

Consumer carbon isotope values also showed spatial heterogeneity, however, patterns and mechanisms differed between consumers. For example, the polychaete *Hediste diversicolor* is predominantly dependent on benthic primary production (Figure 3.3A) even though the species is known to be a scavenger that adapts its diet to food availability including phytoplankton, zooplankton and bacteria (Costa et al. 2006). The bivalve *Limecola balthica* showed differential pelagic-benthic consumption with a high pelagic contribution in some areas of the Wadden Sea (Figure 3.3B). This facultative deposit feeding bivalve (de Goeij et al. 2001) lives buried in the mud and uses its siphon to feed on organic matter from the sediment surface (greenish areas; Figure 3.3B) or in the water (more reddish areas; Figure 3.3B). Spatial heterogeneity patterns in *L. balthica* could not be explained by ontogenetic shifts towards more suspension feeding in larger individuals (Rossi et al. 2004) ($R^2 = 0.001$, Figure S3). The bivalve *Cerastoderma edule* is an obligatory suspension feeder (Kamermans 1994) comparable to the mussels collected from buoys in deeper water. Specifically, it feeds much closer to the sediment-water interface and thus although pelagic primary production dominated the $\delta^{13}C$ values in this species (Figure 3.3C), this species also incorporated resuspended benthic algae.

The different spatial patterning observed for the different benthic consumers likely results from various factors: (1) spatial variability in benthic production, (2) differential consumption of benthic and pelagic producers and/or (3) the differential contribution of resuspended benthic primary producers for consumers feeding entirely on pelagic producers. The spatial heterogeneity at multiple trophic levels emphasizes the necessity of location dependent food-web modelling over large spatial scales. For many species of higher trophic levels, food source contribution estimates have to be interpreted with care and more advanced extrapolation techniques may have to be developed to cope with the low spatial sampling resolution of some species and the considerable movement range of individuals.

Implications for nature conservation

The observation that a substantial portion of the food web depends on local benthic primary production in the Dutch Wadden Sea implies that human and naturally induced disturbance of mudflats, and its benthic diatoms, could have cascading effects further up the food web. Current human activities in the Wadden Sea, such as bottom trawling for shrimp, sand suppletion to reduce coastal erosion, drilling for gas, dredging for shells (including the hand-dredging for cockles), and dredging of shipping routes, all potentially affect benthic productivity, as they modify light availability, sediment grain size, air exposure time, surface area available for benthic primary producers, depth of tidal areas or destroy diatom mats (Beukema 1995, Piersma et al. 2001a, Erftemeijer and Lewis 2006, Eriksson et al. 2010, Mercado-Allen and Goldberg 2011, Compton et al. 2016). Our study underlines the pivotal role of benthic primary producers in this ecosystem and thus the pressing need to preserve and protect these pillars of the food web and the intertidal flats on which they grow. The further inclusion of food web studies and basic food web metrics (Christianen et al. 2016) can support monitoring and management of these ecosystems.

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Supporting information

Table S1: Average δ^{13}C values and their range for 178 species that were collected in the Dutch Wadden Sea between 2008–2012. Contribution of benthic primary producer production is given for species that were sampled at more than one location. The list of species is not complete and the selection of species presented is determined by availability of samples for stable isotope analysis.

Group	Species	Benthic contr.		δ^{13}C		
		av ± SE	n	av ± SE	min	max
Plants	*Zostera marina*		40	−12.8 ± 0.2	−15.2	−11.1
	Zostera noltii		20	−13.2 ± 0.2	−15.1	−10.9
Algae	*Enteromorpha* sp		10	−15.7 ± 0.7	−19.4	−11.6
	Fucus vesiculosus		56	−16.4 ± 0.2	−20.5	−13.1
	Ulva ulva		65	−14.0 ± 0.3	−18.5	−9.2
Annelids	*Alitta succinea*	>95 ± 86	22	−17.2 ± 0.3	−19.2	−12.7
	Alitta virens	10 ± 1	2	−17.6 ± 0.1	−17.7	−17.5
	Aphelochaeta marioni	27 ± 12	4	−17.7 ± 0.4	−18.2	−16.6
	Arenicola marina	50 ± 11	107	−16.4 ± 0.1	−20.7	−14.1
	Blidingia minima	na ± na	1	−20.7 ± na	−20.7	−20.7
	Bylgides sarsi	>95 ± 50	12	−16.9 ± 0.3	−18.3	−15.4
	Capitella capitata	<5 ± 9	5	−17.9 ± 0.2	−18.5	−17.2
	Dipolydora coeca	na ± na	1	−18.8 ± na	−18.8	−18.8
	Eteone longa	>95 ± 11	39	−15.6 ± 0.2	−17.8	−12.6
	Eumida sanguinea	na ± na	1	−17.8 ± na	−17.8	−17.8
	Eunereis longissima	52 ± 37	5	−16.8 ± 0.9	−18.4	−13.5
	Glycera alba	na ± na	1	−18.8 ± na	−18.8	−18.8
	Gracilariopsis longissima	38 ± 23	2	−16.8 ± 1.0	−17.8	−15.8
	Harmothoe imbricata	na ± na	1	−16.6 ± na	−16.6	−16.6
	Harmothoe impar	na ± na	1	−16.5 ± na	−16.5	−16.5
	Hediste diversicolor	>95 ± 9	160	−16.1 ± 0.1	−20.2	−9.9
	Heteromastus filiformis	11 ± 10	20	−17.7 ± 0.3	−19.7	−14.2
	Lanice conchilega	<5 ± 21	40	−18.0 ± 0.2	−20.3	−14.9
	Magelona sp	43 ± 43	2	−18.0 ± 0.3	−18.3	−17.7
	Marenzelleria viridis	<5 ± 19	12	−18.1 ± 0.3	−19.5	−16.0
	Nemertini sp	na ± na	1	−15.0 ± na	−15.0	−15.0
	Nephtys hombergii	>95 ± 44	22	−15.1 ± 0.2	−17.9	−13.8
	Phyllodoce maculata	63 ± 18	10	−17.0 ± 0.4	−19.1	−14.9
	Phyllodoce mucosa	na ± na	1	−18.2 ± na	−18.2	−18.2
	Scolelepis foliosa	55 ± 11	7	−15.5 ± 0.1	−15.8	−15.1
	Scoloplos armiger	71 ± 7	77	−16.4 ± 0.1	−19.5	−13.2
	Spionide sp	>95 ± 35	9	−16.6 ± 0.4	−17.8	−14.2
Crustaceans	*Bathyporeia sarsi*	67 ± 59	2	−14.8 ± 1.9	−16.6	−12.9
	Bathyporeia sp	79 ± 52	6	−14.4 ± 0.5	−17.1	−13.7
	Cancer pagurus	14 ± 5	7	−16.7 ± 0.1	−17.0	−16.3
	Carcinus maenas	>95 ± 8	1077	−15.7 ± 0.0	−21.3	−11.3
	Corophium sp	<5 ± 15	17	−18.3 ± 0.6	−21.5	−12.9
	Crangon crangon	>95 ± 13	358	−15.0 ± 0.1	−23.2	−11.4
	Eriocheir sinensis	<5 ± 17	2	−24.5 ± 3.9	−28.4	−20.6
	Gammarus locusta	<5 ± 30	2	−19.3 ± 1.1	−20.4	−18.2
	Hemigrapsus sanguineus	9 ± 2	4	−17.9 ± 0.2	−18.2	−17.4
	Hemigrapsus takanoi	>95 ± 29	25	−16.1 ± 0.3	−19.5	−14.3
	Hyperia galba	<5 ± 1	14	−18.8 ± 0.2	−20.3	−17.8
	Idotea linearis	23 ± 12	19	−18.3 ± 0.2	−20.3	−16.0
	Liocarcinus holsatus	<5 ± 12	102	−18.0 ± 0.1	−21.9	−14.2
	Macropodia rostrata	<5 ± 3	4	−19.2 ± 0.4	−20.2	−18.5
	Pagurus bernhardus	<5 ± 19	15	−19.3 ± 0.3	−20.6	−16.6

Table S1: Continued.

Group	Species	Benthic contr.		δ13C		
		av ± SE	n	av ± SE	min	max
Crustaceans	*Palaemon adspersus*	67 ± 50	3	−14.3 ± 0.5	−15.2	−13.8
	Palaemon elegans	>95 ± 24	70	−17.3 ± 0.2	−23.8	−13.7
	Palaemon serratus	14 ± 4	8	−16.8 ± 0.4	−18.1	−15.0
	Palaemon sp	10 ± 16	12	−21.9 ± 1.5	−27.4	−12.9
	Pinnotheres pisum	na ± na	1	−21.8 ± na	−21.8	−21.8
	Praunus flexuosus	29 ± 10	74	−16.8 ± 0.2	−26.9	−12.7
	Urothoe poseidonis	68 ± 13	39	−15.9 ± 0.2	−19.0	−11.3
Echinoderms	*Actiniaria* sp	32 ± 10	40	−18.0 ± 0.1	−19.4	−14.6
	Asterias rubens	20 ± 20	54	−17.1 ± 0.2	−20.9	−13.2
	Metridium senile	41 ± 22	29	−18.4 ± 0.2	−20.5	−16.6
	Sagartia sp	28 ± 11	10	−17.7 ± 0.2	−18.8	−16.8
Jellyfish	*Aequorea vitrina*	<5 ± 0	6	−19.0 ± 0.3	−19.5	−18.1
	Aurelia aurita	<5 ± 4	5	−18.9 ± 0.4	−20.3	−18.1
	Beroe cucumis	na ± na	1	−18.0 ± na	−18.0	−18.0
	Chrysaora hysoscella	<5 ± 12	40	−19.3 ± 0.3	−21.8	−13.4
	Clytia hemisphaerica	na ± na	3	−22.2 ± 0.4	−23.0	−21.8
	Cyanea capillata	na ± na	1	−18.5 ± na	−18.5	−18.5
	Cyanea lamarckii	<5 ± 1	7	−18.1 ± 0.3	−18.9	−16.3
	Eucheilota maculata	<5 ± 33	5	−21.3 ± 0.8	−23.1	−19.0
	Mnemiopsis leidyi	<5 ± 3	44	−19.3 ± 0.2	−22.2	−17.2
	Nemopsis bachei	<5 ± 4	26	−18.8 ± 0.2	−21.7	−17.8
	Pleurobrachia pileus	<5 ± 2	33	−18.4 ± 0.3	−21.7	−16.4
	Rhizostoma pulmo	10 ± 14	30	−19.0 ± 0.3	−21.4	−15.2
	Sarsia tubulosa	<5 ± 0	5	−18.6 ± 0.1	−18.8	−18.2
	Tubularia	<5 ± 13	2	−19.3 ± 0.7	−19.9	−18.6
Molluscs	*Abra alba*	6 ± 12	2	−17.3 ± 0.1	−17.4	−17.2
	Abra tenuis	>95 ± 26	18	−13.2 ± 0.6	−16.5	−6.4
	Alloteuthis subulata	<5 ± 5	2	−19.1 ± 0.5	−19.6	−18.6
	Balanus crenatus	19 ± 6	81	−18.3 ± 0.2	−23.5	−13.8
	Cerastoderma edule	<5 ± 4	381	−18.7 ± 0.1	−21.9	−6.5
	Crassostrea gigas	16 ± 13	37	−17.8 ± 0.1	−20.8	−16.8
	Crepidula fornicata	21 ± 11	34	−17.7 ± 0.1	−19.2	−16.6
	Elminius modestus	na ± na	1	−19.2 ± na	−19.2	−19.2
	Ensis directus	17 ± 9	55	−18.2 ± 0.2	−21.0	−11.8
	Kurtiella bidentata	na ± na	1	−17.7 ± na	−17.7	−17.7
	Lepidochitona cinerea	>95 ± 41	18	−13.4 ± 0.5	−17.0	−8.9
	Littorina littorea	>95 ± 25	60	−14.2 ± 0.2	−17.1	−10.6
	Loligo vulgaris	na ± na	1	−18.4 ± na	−18.4	−18.4
	Macoma balthica	>95 ± 11	178	−16.0 ± 0.1	−20.9	−7.9
	Mactra corallina	54 ± 4	3	−16.1 ± 0.2	−16.4	−15.9
	Mya arenaria	53 ± 18	47	−17.2 ± 0.5	−21.1	−3.4
	Mytilus edulis	38 ± 14	263	−18.2 ± 0.1	−23.5	−9.0
	Petricolaria pholadiformis	58 ± 54	7	−18.1 ± 0.2	−19.0	−17.2
	Retusa obtusa	>95 ± 50	11	−14.5 ± 1.1	−18.1	−7.4
	Scrobicularia plana	>95 ± 38	22	−15.7 ± 0.4	−19.7	−11.1
	Sepiola atlantica	na ± na	1	−18.4 ± na	−18.4	−18.4
	Sessilia sp	24 ± 20	12	−17.7 ± 0.3	−19.2	−16.5
	Tellina tenuis	na ± na	1	−16.5 ± na	−16.5	−16.5
	Ventrosia ventrosa	19 ± 1	2	−16.0 ± 0.0	−16.0	−16.0
Fish	*Agonus cataphractus*	19 ± 6	17	−17.0 ± 0.2	−18.6	−16.0
	Alosa fallax	14 ± 17	43	−19.4 ± 0.3	−24.0	−15.1
	Ammodytes tobianus	10 ± 3	31	−18.1 ± 0.2	−20.7	−16.7
	Anguilla anguilla	18 ± 26	5	−19.4 ± 3.3	−32.6	−14.9
	Aphia minuta	<5 ± 4	4	−19.3 ± 0.6	−20.8	−18.1
	Arnoglossus laterna	<5 ± 15	5	−18.7 ± 0.3	−19.5	−17.8

Table S1: Continued.

Group	Species	Benthic contr.				δ^{13}C				
		av	±	SE	n	av	±	SE	min	max
Fish	*Atherina presbyter*	18	±	2	3	−16.0	±	0.4	−16.7	−15.5
	Belone belone	<5	±	3	18	−18.0	±	0.3	−20.7	−16.8
	Callionymus lyra	na	±	na	1	−18.9	±	na	−18.9	−18.9
	Callionymus reticulatus	na	±	na	1	−18.3	±	na	−18.3	−18.3
	Centrolabrus exoletus	29	±	21	20	−17.8	±	0.4	−22.3	−15.3
	Chelon labrosus	77	±	26	132	−16.1	±	0.2	−25.6	−10.0
	Ciliata mustela	27	±	9	47	−17.0	±	0.2	−20.5	−14.6
	Clupea harengus	<5	±	11	250	−18.9	±	0.1	−29.8	−16.5
	Coregonus oxyrinchus	na	±	na	1	−19.5	±	na	−19.5	−19.5
	Cyclopterus lumpus	<5	±	3	5	−19.3	±	0.5	−20.5	−17.9
	Dicentrarchus labrax	12	±	2	119	−16.7	±	0.2	−21.2	−12.4
	Echiichthys vipera	6	±	11	3	−18.2	±	0.4	−18.7	−17.4
	Engraulis encrasicolus	<5	±	2	12	−19.2	±	0.3	−19.9	−17.2
	Eutrigla gurnardus	na	±	na	1	−15.5	±	na	−15.5	−15.5
	Gadus morhua	12	±	2	28	−16.5	±	0.1	−18.1	−15.2
	Gasterosteus aculeatus	<5	±	34	97	−20.9	±	0.3	−32.8	−16.3
	Gastrosaccus spinifer	<5	±	2	15	−19.9	±	0.2	−21.3	−18.5
	Hyperoplus lanceolatus	<5	±	2	3	−18.9	±	0.4	−19.6	−18.4
	Katsuwonus pelamis	<5	±	6	4	−21.8	±	0.4	−22.4	−21.0
	Lampetra fluviatilis	<5	±	19	3	−23.3	±	2.2	−27.5	−20.3
	Limanda limanda	9	±	2	26	−17.8	±	0.2	−19.1	−14.9
	Liparis liparis	27	±	16	56	−16.7	±	0.1	−18.9	−14.6
	Liza aurata	17	±	15	14	−13.4	±	1.0	−19.7	−8.9
	Liza ramada	6	±	8	6	−17.9	±	0.8	−20.2	−15.6
	Merlangius merlangus	10	±	8	85	−17.4	±	0.1	−19.9	−15.7
	Microstomus kitt	8	±	16	2	−17.6	±	0.3	−18.0	−17.3
	Mullus surmuletus	<5	±	40	3	−19.2	±	1.1	−21.1	−17.3
	Myoxocephalus scorpius	70	±	22	35	−16.1	±	0.1	−17.4	−15.0
	Osmerus eperlanus	74	±	21	159	−18.1	±	0.2	−27.9	−12.7
	Petromyzon marinus	na	±	na	1	−19.5	±	na	−19.5	−19.5
	Pholis gunnellus	65	±	50	18	−17.5	±	0.2	−18.6	−16.1
	Phycis blennoides	na	±	na	1	−18.0	±	na	−18.0	−18.0
	Platichthys flesus	>95	±	21	167	−16.9	±	0.2	−27.4	−12.3
	Pleuronectes platessa	58	±	12	202	−16.2	±	0.1	−21.9	−13.1
	Pollachius pollachius	58	±	42	12	−16.2	±	0.3	−17.6	−14.0
	Pollachius virens	7	±	2	11	−18.0	±	0.4	−20.2	−16.1
	Pomatoschistus lozanoi	6	±	2	49	−17.5	±	0.1	−21.2	−16.4
	Pomatoschistus microps	32	±	11	57	−16.3	±	0.2	−21.2	−13.1
	Pomatoschistus minutus	33	±	9	136	−17.1	±	0.1	−21.4	−14.5
	Pomatoschistus pictus	na	±	na	1	−17.9	±	na	−17.9	−17.9
	Salmo trutta	<5	±	2	28	−18.6	±	0.3	−24.5	−17.2
	Sardina pilchardus	8	±	11	22	−19.0	±	0.4	−22.3	−16.7
	Scomber scombrus	<5	±	4	5	−19.4	±	0.7	−21.2	−17.5
	Scophthalmus maximus	15	±	4	4	−15.9	±	0.8	−17.3	−13.6
	Scophthalmus rhombus	25	±	4	3	−14.7	±	0.6	−15.6	−13.6
	Solea solea	86	±	22	45	−16.7	±	0.1	−19.1	−14.6
	Sprattus sprattus	<5	±	4	69	−18.8	±	0.1	−21.6	−17.2
	Syngnathus acus	10	±	1	8	−17.2	±	0.2	−17.8	−16.6
	Syngnathus rostellatus	11	±	3	136	−17.9	±	0.1	−20.6	−14.5
	Taurulus bubalis	na	±	na	1	−19.5	±	na	−19.5	−19.5
	Thunnus thynnus	14	±	1	3	−17.2	±	0.4	−17.7	−16.3
	Trachurus trachurus	<5	±	14	35	−19.0	±	0.3	−21.7	−15.0
	Trigla lucerna	38	±	28	20	−16.8	±	0.3	−20.0	−14.9
	Trisopterus luscus	7	±	4	7	−17.5	±	0.2	−18.8	−16.8
	Zoarces viviparus	78	±	21	49	−16.1	±	0.2	−19.1	−13.9

Table S1: Continued.

Group	Species	Benthic contr.		$\delta^{13}C$		
		av ± SE	n	av ± SE	min	max
Birds	*Branta bernicla*	<5 ± 4	23	−25.1 ± 0.2	−27.8	−22.9
	Calidris alba	79 ± 48	142	−18.5 ± 0.2	−24.3	−13.5
	Calidris alpina	56 ± 28	169	−16.1 ± 0.2	−27.7	−13.3
	Calidris canutus	59 ± 35	194	−18.0 ± 0.1	−26.8	−14.9
	Calidris ferruginea	9 ± 20	2	−16.8 ± 0.6	−17.4	−16.2
	Chroicocephalus ridibundus	na ± na	11	−16.6 ± 0.4	−18.3	−14.8
	Limosa lapponica	79 ± 37	44	−17.3 ± 0.4	−26.9	−14.1
	Platalea leucorodia	<5 ± 1	935	−18.4 ± 0.1	−30.6	−13.6
	Recurvirostra avosetta	36 ± 15	20	−17.7 ± 0.3	−19.2	−14.9
	Sterna hirundo	na ± na	3	−15.6 ± 0.2	−15.9	−15.3
	Sterna sandvicensis	na ± na	6	−15.2 ± 0.1	−15.4	−14.9
Mammals	*Phoca vitulina*	11 ± 3	67	−15.9 ± 0.1	−17.9	−13.7
	Phocoena phocoena	49 ± 18	23	−18.2 ± 0.1	−19.2	−17.4
Other	benthic primariy producers	>95 ± 13	229	−14.8 ± 0.2	−21.7	−9.9
	SOM	19 ± 27	25	−18.9 ± 0.3	−22.2	−17.4
	wPOM	<5 ± 4	66	−18.9 ± 0.2	−22.5	−15.7
	Zooplankton	15 ± 8	29	−18.4 ± 0.2	−21.1	−13.8
	Sertularia cupressina	na ± na	1	−19.8 ± na	−19.8	−19.8
	Urticina felina	8 ± 11	3	−17.8 ± 0.0	−17.9	−17.8
	Didemnum lahillei	<5 ± 9	3	−18.3 ± 0.2	−18.7	−18.0

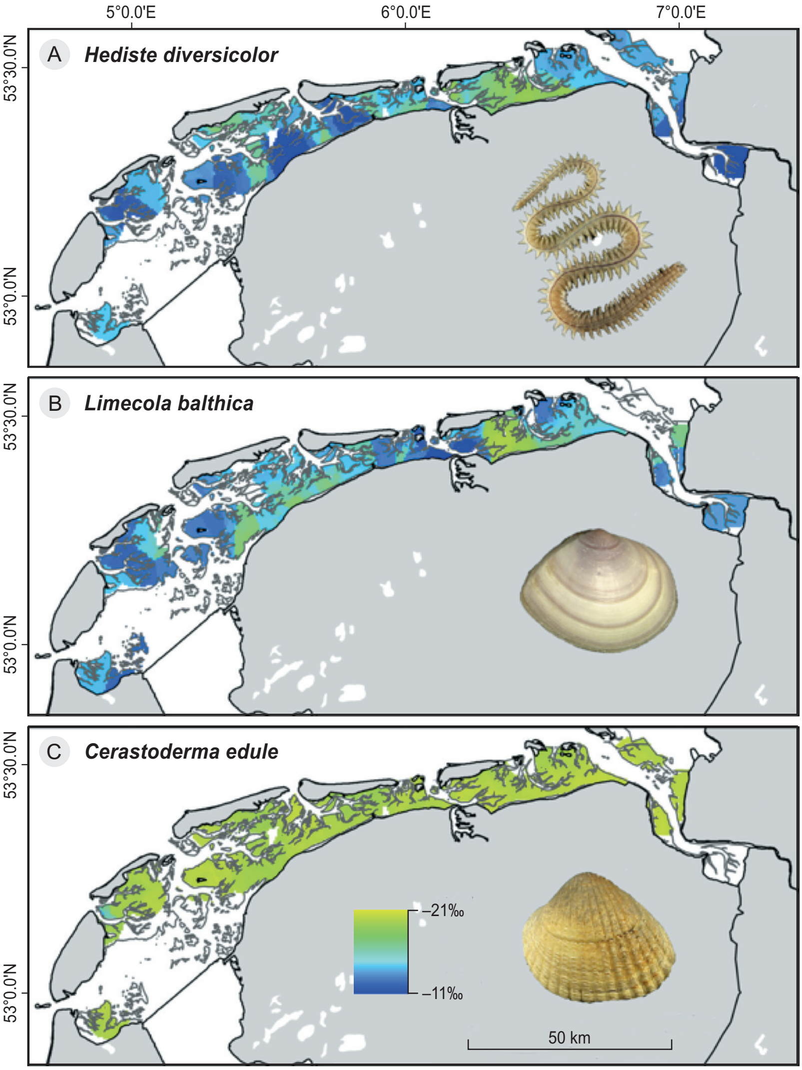


Figure S2: Map of $\delta^{13}C$ values of consumers (A) *Hediste diversicolor* (n = 120), (B) *Limecola balthica* (n = 139), (C) *Cerastoderma edule* (n = 346), extrapolated over the Dutch Wadden Sea. $\delta^{13}C$ values of consumers reflect that of their food source and stay rather constant throughout the food chain. $\delta^{13}C$ values can therefore be used as a tracer to analyze the contribution of the different primary producers to the production the organisms in a food web. The color scale used to depict $\delta^{13}C$ values matches the colors used in other figures.

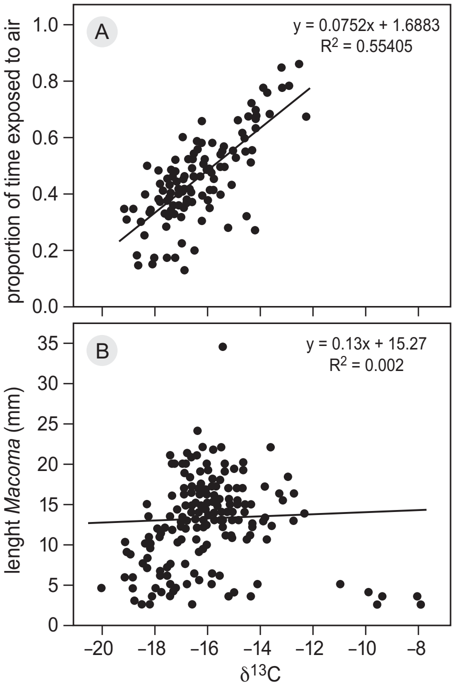


Figure S3: Correlation between $\delta^{13}C$ values and (A) time exposed to air on intertidal areas for *Peringia ulvae* (proxy of benthic primary producers) (n = 126), (B) size for *Limecola balthica* (n = 271). An ontogenetic shift in foraging strategy with increasing size of *L. balthica* seems absent.

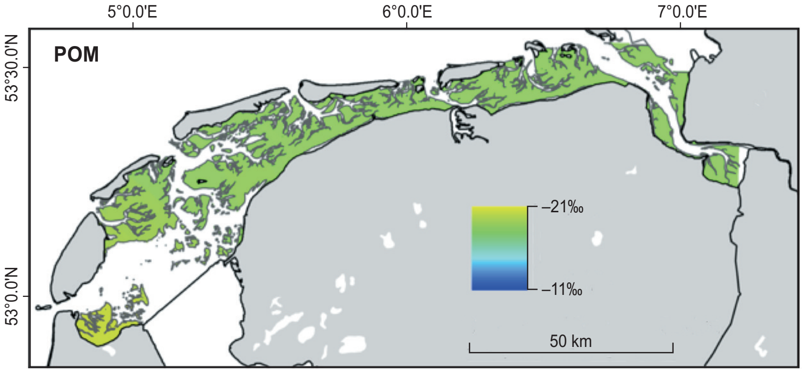
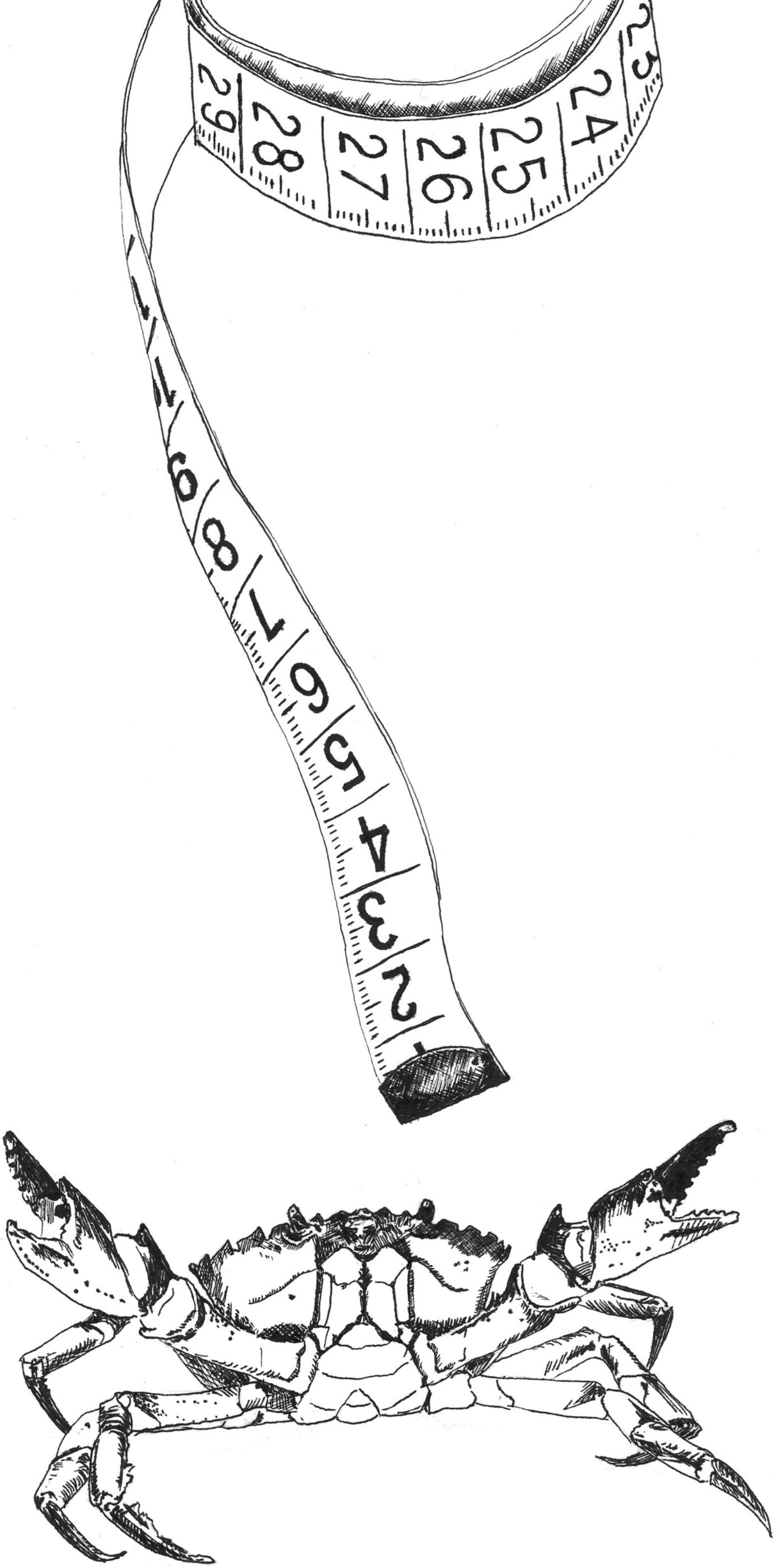


Figure S4: Map of δ^{13}C values of particulate organic matter in the water column (n = 73) extrapolated over the Dutch Wadden Sea. The color scale used to depict δ^{13}C values matches the colors used in other figures.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CHAPTER 4

Spatial heterogeneity in coastal stable nitrogen isotope baselines, does it matter?

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Sander J. Holthuijsen, Stefan Schouten & Laura L. Govers

Abstract

The analysis of nitrogen stable isotopes ($\delta^{15}N$) for different species is a powerful, frequently used method to reconstruct food webs that are increasingly sampled at higher spatial resolution within ecosystems. With the correct baseline, these isotope values indicate the average trophic position of species, but may also reveal spatial heterogeneity in food web structure. However, when spatial heterogeneity in food web structure is of interest, the assumption of a single baseline value for all sampling locations may be inappropriate, especially for sedentary species. Instead, the calculation of a spatially explicit trophic baseline is then needed, which is rarely done. Especially for somewhat more mobile species. it is not always possible or justified to take baseline samples only at the position where the food web sample has been taken. Either the species may have foraged elsewhere, or its food may have been moved due to water currents. For this, interpolations of sampled baselines could offer a solution but this calculations of spatial heterogeneity in trophic position sensitive to the interpolation methods used, in combination with assumptions on the mobility of the species involved.

For the food web of the intertidal Dutch Wadden Sea, we explored the measurement and consequences of spatial baseline heterogeneity on the trophic positions for species with different mobility (small/large spatial reach) and food source (benthic/pelagic). Covering a large ecosystem extent (~ 2500 km²), we sampled with high spatial resolution four species of different trophic level and mobility (shorecrab *Carcinus maenas*, mullet *Chelon labrosus*, barnacle *Balanus crenatus* and herring *Clupea harengus*) and reckon their food source for baseline selection. We explored different interpolation methods for baseline values with and without abiotic environmental factors as covariates, and at different spatial scales. We show that the importance of taking baseline heterogeneity into account for trophic level calculation highly differed among species. The benthic low mobility shore crab, the benthic higher mobility thick-lipped mullet, and the mobile, pelagic feeding Atlantic herring were relatively insensitive to baseline heterogeneity as determined by the different methods. In contrast, the sessile zooplankton feeding barnacle showed strong sensitivity to spatial baseline heterogeneity of medium- and small scale interpolated measured baseline values, especially in the Eems-Dollard estuary. We conclude that taking species-specific sensitivity to baseline heterogeneity is an important step in unravelling the spatial structure of food webs.

Manuscript

Introduction

Stable isotopes have become a widely used and powerful tool for food web reconstruction in trophic ecology (Arbi, Liu, Zhang et al. 2018; Robert & Pawlowski 2018; Chouvelon et al. 2012; Post 2002; Kopp, Peterson & Fry 1987). Stable nitrogen isotopes ($\delta^{15}N$) are particularly interesting since they can be used to estimate the trophic position of consumers, based on a typical enrichment of ~3.4‰ in $\delta^{15}N$ per trophic level relative to the $\delta^{15}N$ of the food source (Solomon et al. 2008; Anderson & Cabana 2007; Post 2002), making the $\delta^{15}N$ value at the base of the food web important for correct calculation of trophic positons. An appropriate isotopic baseline is thus essential for studies in trophic ecology (Chouvelon et al. 2012; Post 2002).

But how can we determine a proper baseline? For the reconstruction of a general food web, a single spatially integrated baseline value, often the mean $\delta^{15}N$-value of multiple baseline samples, may be sufficient (Chouvelon et al. 2012; Hansen et al. 2012; Gibb & Cunningham 2011; Post 2002). However, taking spatial heterogeneity of a baseline into account may be important when trophic positions of consumers vary among sites, not only as a function of their trophic position, but also due to spatial heterogeneity of the baseline (Chouvelon et al. 2012). In marine ecosystems, this spatial variation may be due to for instance environmental factors such as seafloor temperature, nutrient availability, salinity and depth (Hanson, Jones & Harris 2018; Barnes et al. 2009; Jennings & Warr 2003; Mullin et al. 1984). These factors all affect $\delta^{15}N$-values of primary producers through variation in nitrogen source, fixation and decomposition processes (Peterson & Fry 1987). Reckoning spatial baseline heterogeneity, when estimating trophic positions of consumers, is also important for finding indicator species for food web structure across landscape gradients (Jordán 2009; Vander Zanden et al. 1999a). Additionally, food web reconstructions are conducted on increasingly finer spatial resolutions within ecosystems (Rumolo et al. 2016; Hansen et al. 2012; Guzzo et al. 2011). Such resolutions also require the use of a spatial explicit baseline. Moreover, the need for a spatially explicit baseline for trophic level estimations may vary among species within a single system. This may be attributed to the ability of highly mobile species to integrate spatial heterogeneity in $\delta^{15}N$ signals across a landscape-scale, whereas species with limited mobility do not integrate such mobility (Lehmitz & Maraun 2016; Cronin et al. 2015; Woodcock et al. 2012; Anderson & Cabana 2007).

The importance of addressing spatial variation in stable isotope baselines to enable spatially explicit food web reconstructions is more and more recognized and applied (Hanson, Jones & Harris 2018;Rumolo et al. 2016; Kurten et al. 2013; Hansen et al. 2012; Guzzo et al. 2011; Barnes et al. 2009; Jennings & Warr 2003). This is for instance being done by sampling a baseline value at each consumer sampling location. However, for practical reasons, this intensive method of baseline sampling is not always feasible as baseline species may not be present at every single sampling point or logistical or financial limitations may apply in large-scale systems (>50 km). Other challenges using this sampling technique are high mobility of a consumer species that feeds elsewhere and local baseline heterogeneity due to highly variable $\delta^{15}N$ -values of the baseline species as a result of life stage, size class or feeding mode (Brandstrator et al. 2000; Vanderklift & Ponsard 2003). This causes a single baseline (prey species) sample to be insufficiently representative of the individual home range of the focal consumer. The necessity to adjust for feeding mode of focal consumers by the use of an appropriate baseline that suits the feeding mode, is becoming generally accepted (Anderson & Cabana 2007). For instance, coastal ecosystems can be driven by both benthic and pelagic primary producers with distinct stable isotope values (both $\delta^{13}C$ and $\delta^{15}N$), which is reflected by their consumers (Christianen et al. 2017a). Potential variation in $\delta^{15}N$ values between such primary producers may lead to over- or underestimation of trophic positions of higher consumers when not taken into account.

In coastal ecosystems, the effects of spatial baseline heterogeneity on trophic level of consumers is so far only studied mostly on a coarse spatial scales, e.g., 60–70 samples per 700,000 km² (Jennings & Warr 2003; Barnes et al. 2009) Finer-scale information on baseline $\delta^{15}N$ is rarely available (Jennings & Warr 2003). However, baseline $\delta^{15}N$ levels may also be highly variable on this scale, as is known from terrestrial ecosystems (Lehmitz & Maraun 2016; Woodcock et al. 2012; Cronin et al. 2015). At this point it is unclear if the higher mobility of marine organisms at the base of the food web really justifies the assumption of spatially uniform baselines.

In this study we explore if such baseline heterogeneity exists on a smaller spatial scale (>100 samples per 2500 km²) in a densely sampled coastal area (Dutch Wadden Sea) and if observed spatial heterogeneity in trophic levels of consumers are due to baseline heterogeneity or actual spatial differences in consumer trophic positions (food web complexity). More specifically, we aimed to provide general insights in how to deal with spatial variation in $\delta^{15}N$ baselines in marine trophic ecology, taking both consumer mobility and general feeding mode into account. We examined 1) if spatial heterogeneity in $\delta^{15}N$ baselines should be taken into account for trophic level estimations of consumers in a highly-connected coastal ecosystem and 2) if consumers' mobility affects the need for the use of a spatially explicit baseline. We hypothesize that spatial variation in a consumer's trophic level may be the result of a) spatial heterogeneity of the baseline, b) spatial homogeneity of the baseline but actual spatial heterogeneity of consumer's trophic levels or c) heterogeneity in both the baseline and the actual consumer's trophic level (Figure 4.1).

To answer these questions, we constructed spatial baselines of two feeding modes, using $\delta^{15}N$ values of a benthic primary consumer (the microphytobentos grazer *Peringia ulvae*) and a pelagic filter feeding primary consumer (*Mytilus edulis*). The advantage of using these consumers as baselines rather than direct sampling of algae,

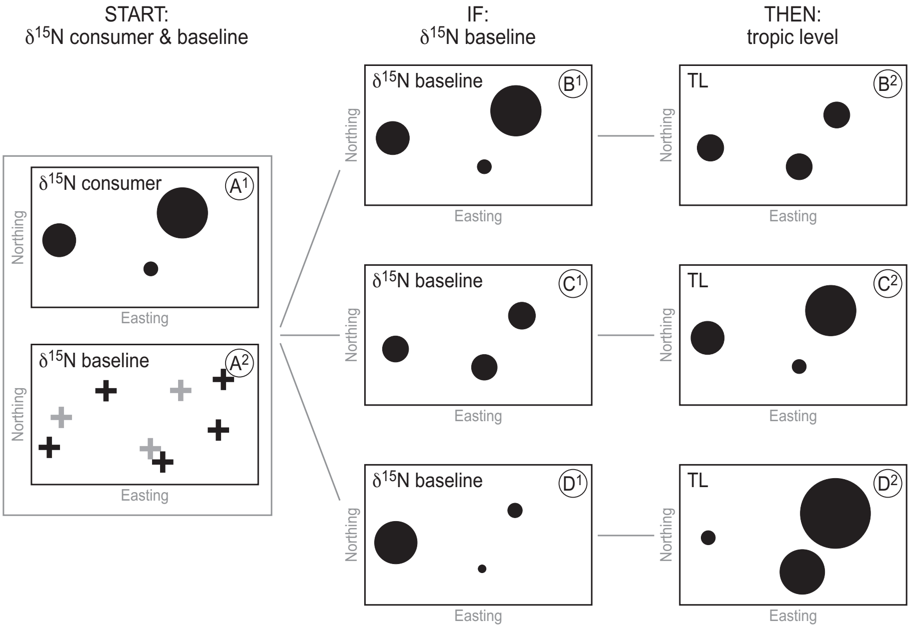


Figure 4.1: Hypothetical explanations for spatial variation in consumer's trophic levels. Spatial homogeneity or heterogeneity in consumer's trophic levels may be explained by B) similar spatial heterogeneity in baseline δ^{15}N as consumer δ^{15}N resulting in a spatially homogeneous consumer's trophic level, a dissimilarity in spatial variation between consumer δ^{15}N and baseline δ^{15}N through C) a homogeneous baseline resulting in actual spatial heterogeneity of trophic levels or D) a complete mismatch in consumer and baseline δ^{15}N, also resulting in actual spatial heterogeneity of trophic levels. Circle size illustrates the hypothetical value at that position and crosses indicate hypothetical sampling sites of baseline species.

is better integration over short-term variability in isotope signals. Subsequently, we constructed interpolated trophic baselines for both feeding modes, varying in spatial scale and approach, using (1) mean δ^{15}N values for the whole Dutch Wadden Sea area to calculate one, homogenized baseline value representing a large spatial scale baseline, (2) a large-scale kriging extrapolation of measured δ^{15}N values to create a baseline with a 'measured medium spatial scale baseline', (3) a small-scale kriging extrapolation of measured δ^{15}N values to create a 'measured small spatial scale baseline', (4) a medium-scale interpolation of model-predicted δ^{15}N values to create 'modeled medium spatial scale baseline' and (5) a small-scale interpolation of model-predicted δ^{15}N values to create a 'modeled small spatial scale baseline'. We tested the effects of these five different approaches on trophic level calculations on four model consumer species with a either a benthic or pelagic feeding mode and with a low or high mobility (shore crab *Carcinus maenas* (Benthic feeding, Low mobility), thick-lipped mullet

Chelon labrosus (Benthic feeding, High mobility), barnacle *Balanus crenates* (Pelagic feeding, Low mobility) and herring *Clupea harengus* (Pelagic, High mobility)).

Materials and Methods

Study system

The Dutch Wadden Sea encompasses an area of around 2500 km^2, of which around 1460 km^2 of intertidal mudflats (de Jonge et al. 1993; Wolff 2000a). The entire Wadden Sea area spans from The Netherlands to Denmark and covers 8000 km^2. The (Dutch) Wadden Sea is connected to freshwater via inlets from the mainland and adjacent barrier islands and to connections with the North Sea via inlets between the barrier islands (Figure 4.2) (Compton et al. 2013; Wolff 2000b). The Wadden Sea is heavily modified by human activities, both in terms of geomorphology (e.g. Wolff 1983) and resource depletion (e.g. Eriksson et al. 2010; Lotze et al. 2006), which – as a consequence – led to an assembly of degraded states over a large spatial extent. However, up to date the Wadden sea is of crucial importance for many species and is a key foraging and resting site along the Atlantic flyway for migratory shorebirds (Blew & Südbeck 2005; Compton et al. 2013; Reise et al. 2010; van Roomen et al. 2012). The food web of the shallow Wadden Sea ecosystem is mainly fuelled by benthic rather than by pelagic algae. Recent research showed that 74% of all benthic species relies either directly or indirectly on benthic primary producers as a carbon source (Christianen et al. 2017). Nitrogen has several pathways into the food web: 1) nitrogen fixation by bacteria and archaea, 2) uptake of ammonium after organic matter remineralization and, 3) assimilation of dissolved seawater or pore water ammonium or nitrate. The nitrogen source of primary producers is reflected by their $\delta^{15}N$ levels, with benthic and pelagic primary producers respectively fixate atmospheric nitrogen (N_2) with help of bacteria or fixate dissolved nitrogen ions (NO_3^- and NH_3) (Joye & Anderson 2008).

Selection of baseline species

Primary consumers – and not primary producers – are considered to be the best baseline species, since they buffer the short term fluctuations of the short living and highly temporal variable primary producers (Kopp et al. 2015; Post 2002; Cabana & Rasmussen 1996). We therefore selected two common primary consumer species with a low mobility that are either primarily benthic (*Peringia ulvae*) or pelagic (*Mytilus edulis* on floating buoys) feeding species, using $\delta^{13}C$ values to determine feeding mode (see (Christianen et al. 2017a).

Selection of focal consumer species and data collection

We selected four focal consumer species, varying in mobility (low or high) and food source (benthic or pelagic origin). Shore Crabs *Carcinus maenas* were chosen as a species that forages mainly on benthic prey and has a low mobility, with part of the individuals moving with the tide but foraging locally on banks where we caught them

Figure 4.2: A). The position of the Netherlands in northwestern Europe with the Dutch Wadden Sea indicated by the black box and B). The Dutch Wadden Sea, separated from the North Sea by barrier islands.

(Crothers 1967). We selected Crabs of >15mm since $\delta^{15}N$ of crabs <15 mm showed a positive allometric relationship with $\delta^{15}N$. Crabs (n = 746) were caught with pot traps or little fykes on intertidal banks from March until November with main collection during summer in 2011–2013. Adult Thick-lipped Mullets *Chelon labrosus* were chosen as benthic foraging species with a high mobility. Mullets (n = 62) were caught by fishermen from early June to mid-October in 2012 and from late June to mid-September in 2013 with passive fish traps and had a length between 385 – 687 mm. Barnacles *Balanus crenatus* were chosen as a pelagic foraging species with a low mobility (sessile). Barnacles (n = 72) were collected on 1 September 2012 from floating buoys, the same buoys as where we caught *Mytilus edulis* for the pelagic baseline. Atlantic Herring *Clupea harengus* (n = 207) were caught by with various sampling techniques and individual fish length varied between 42 – 252 mm from March until December in 2012–2013. All individuals of the four species were stored at −20°C, then muscle tissue was obtained, the samples were freeze-dried and grounded before stable isotope analysis.

We collected 135 mud snails, *Peringia ulvae*, on 126 sites throughout the Dutch Wadden Sea from June – September 2011 sampled by the Synoptic Intertidal Benthic Survey (SIBES) programme, using 25 cm deep sediment cores (Compton et al. 2013; Bijleveld et al. 2012). In addition, we collected blue mussels, *Mytilus edulis*, on 41 sites in August-September 2012 from floating buoys along waterways in the Wadden Sea to minimize the input of resuspended benthic material. All samples were stored at −20°C before further analysis.

Sample analysis

In the lab, we prepared the *M. edulis* foot muscle tissue for the largest individuals and complete soft tissue for the smallest individuals. *P. ulvae* individuals were analysed as a whole. We prepared muscle tissue of *C. maenas* normally from the claw(s) and sometimes needed to complement this with muscle tissue from legs and carapace. Muscle tissue of *C. labrosus* and *C. harengus* was prepared from the body between the dorsal fin and the lateral line. We also prepared only muscle tissue of *B. crenatus*. Samples were subsequently rinsed with demineralized water, freeze-dried, ground and decalcified (by adding HCl) if required. Homogenized samples, of both baseline samples and consumer samples, were weighed into tin cups and analysed for nitrogen stable isotope composition with a Flash 2000 elemental analyser coupled online with a Delta V Advantage-isotope ratio monitoring mass spectrometer (IRMS, Thermo Scientific). Nitrogen isotope ratios are expressed in the delta (δ) notation ($\delta^{15}N$) relative to Vienna Pee Dee Belemnite (VPDB). Isotope values were calibrated to a laboratory acetanilide standard ($\delta^{15}N$ −1.3 ‰ calibrated on IAEA-N1) and corrected for blank contribution.

Spatial analysis

Spatial baselines of $\delta^{15}N$ values were developed using 5 different interpolation techniques in ArcGIS 10.3. Methodology was similar for both the benthic (*P. ulvae*) and the pelagic (*M. edulis*) baseline. First, we constructed a spatially homogeneous baseline using the mean $\delta^{15}N$ values of each species (n = 41 for *M. edulis* and n = 135 for *P. ulvae*), where spatial heterogeneity between sites was not taken into account. We call this a 'large spatial scale' trophic baseline. Second, we constructed a spatial baseline based on interpolated, sampled ('measured') $\delta^{15}N$ values from our Wadden Sea-wide dataset. Spatial baseline reconstruction was done by interpolation using the Kriging function in in ArcGIS, based on a semi-variogram model. The output cell size was set at 500 m to match the sampling grid size of the Synoptic Benthic Survey (SIBES) programme and we conducted both a small-scale and medium-scale extrapolation, by obtaining the extrapolated cell values from 7 closest sampling points (small-scale) with a maximum range of 10 kilometres or the 25 closest sampling points (medium-scale) with a maximum range of 25 kilometers. These two extrapolation scales represent the movement of sessile organisms or organisms with a low mobility (10 kilometers – small spatial scale) and mobile organisms operating on a medium spatial scale (25 kilometers – medium spatial scale). Lastly, we used multiple linear regression (MLR) models to investigate if we could explain and predict spatial heterogeneity of Wadden Sea baselines using abiotic factors (abiotic factors were obtained by Gräwe et al. 2015). We obtained spatial maps of the following abiotic factors for MLR analysis: sediment grain size, sediment erosion, orbital velocity, exposure time, salinity in rain season, mean salinity, depth, distance to the nearest mussel bed, distance to the nearest gully, distance to the nearest delta and the distance to the nearest source of freshwater. For each individual sampling point, we then determined the value of all these factors for that specific point by using the Values to Points Tool in ArcGIS. Next, we calculated the distance to mussel bed, distance to freshwater, distance to the gulley and distance to North Sea inlet for each individual sampling point through the Near tool in ArcGIS. We then used a backward stepwise multiple linear regression in which we first included all abiotic factors. The final models were the most reduced models that were not significantly worse ($P > 0.05$) than the full model that included all factors. Model selection was based on the Akaike Information Criterion (AIC). Final models were also tested for independence with the Durbin-Watson test and collinearity was checked using the variance inflation factor (VIF) (Field et al. 2012). Next, we created 10,000 random points in the Wadden Sea using the Fish Net Tool in ArcGIS. By the use of the Values to Point tools, we subsequently coupled the local conditions extracted from the abiotic factors maps to the random points. Using the final models from the multiple linear regression analyses (Table S1 and S2), we then calculated the modelled $\delta^{15}N$ value for each random point. These values were subsequently interpolated for the whole Wadden Sea area using the Inverse Distance Weighing Tool in ArcGIS with a maximum distance of 10 km for the small-scale extrapolation and a distance of 25 km for the medium-scale extrapolation.

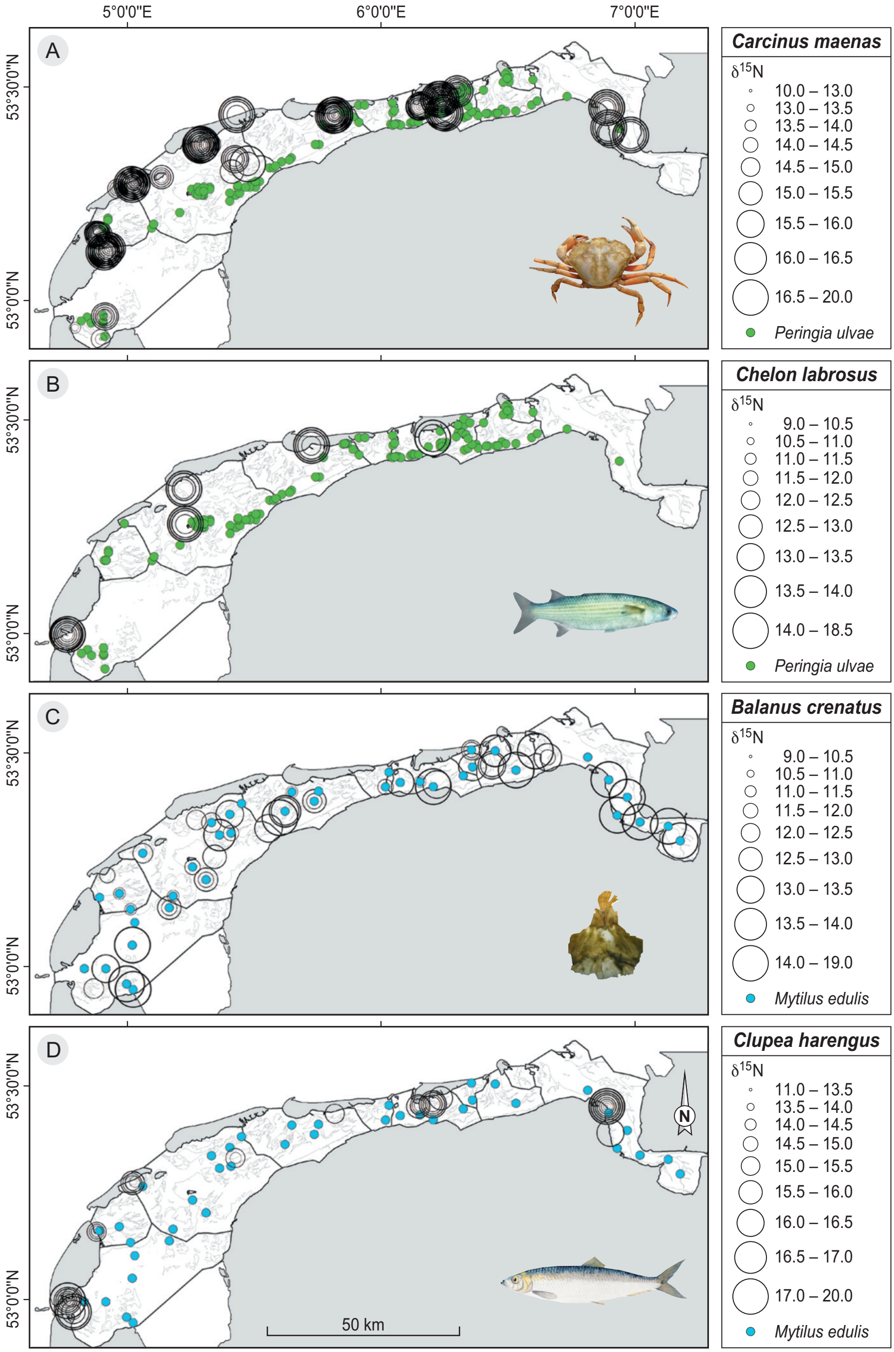
Using these interpolated baseline maps, we calculated the δ^{15}N value for each sample point of our focal consumer species (*Carcinus maenas*, *Chelon labrosus*, *Balanus crenatus*, *Clupea harengus*) using the Multiple Values to Points tool in ArcGIS. Next, the attribute tables containing these values were exported and these values were used to calculate trophic levels (TL) of each sampled focal consumer species by using the following formula (Guzzo et al. 2011) that includes 3.4 as a general fractionation level:

$$\text{Trophic Level (TL)} = \frac{(\delta^{15}\text{N}_{\text{focal consumer}} - \delta^{15}\text{N}_{\text{primary consumer}})}{3.4} + 2$$

For the sensitivity analysis, we used the same extracted values to calculate the absolute differences between the measured δ^{15}N of the consumers and the δ^{15}N values of the five selected baselines. Subsequently, these value differences were averaged per location to prevent inclusion of within site effects and then standard errors of these averaged values were calculated as a measure of sensitivity to baseline heterogeneity. The more similar the standard errors between baselines, the more insensitive a focal species seems for baseline heterogeneity.

Results

For the four higher trophic level consumers, we found substantial variability in δ^{15}N values (Figure 4.3) Shore crab, *C. maenas* (>1.5 cm) showed high within-site variation of δ^{15}N but did not display a clear between-site differences in δ^{15}N across the Dutch Wadden Sea (Figure 4.3A). Similarly, thick-lipped mullet, *C. labrosus* also did not display clear spatial differences in δ^{15}N (Figure 4.3B). In contrast, the barnacle *B. crenatus* showed clear spatial heterogeneity in δ^{15}N with generally lower δ^{15}N values (<12.5‰ δ^{15}N) in the north-western Wadden Sea and with the highest δ^{15}N values in the Eems-Dollard Estuary (>14‰ δ^{15}N) (Figure 4.3C). Herring, *C. harengus*, also showed high within-site variability in addition to distinctly lower δ^{15}N values (<15‰ δ^{15}N) in the north-western Wadden Sea (Figure 4.3D). We questioned whether these spatial patterns in δ^{15}N values of secondary consumers were the result of baseline heterogeneity or an indication of actual spatial variation in trophic levels. Based on Figure 4.3, we hypothesize that taking baseline spatial heterogeneity into account for calculation of trophic levels is only needed for consumers that show higher between-site than within-site variation of δ^{15}N values (e.g. *B. crenatus* and *C. harengus* in Figure 4.3C-D).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Peringia ulvae
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Balanus crenatus
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Mytilus edulis
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Mytilus edulis
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50 km

To characterized baseline spatial heterogeneity with five different methods/ assumptions: (1) mean $\delta^{15}N$ values for the entire Dutch Wadden Sea (large-scale baseline), (2) medium-scale kriging interpolation (25 km) based on measured $\delta^{15}N$ values (medium-scale measured baseline), (3) medium-scale kriging interpolation based on modelled $\delta^{15}N$ values (medium-scale modelled baseline), (4) small-scale kriging interpolation (10 km) based on measured $\delta^{15}N$ values (small-scale measured baseline) and (5) small-scale kriging interpolation based on modelled $\delta^{15}N$ values (small-scale modelled baseline). These methods assume in this order increasing sedentary consumers and their food, where we applied baselines for benthic primary produces using the mudsnail *Peringia ulvae* and for pelagic primary producers using the mussel *Mytilus edulis*.

We found that $\delta^{15}N$ values for *P. ulvae* in the Dutch Wadden Sea ranged from 4.8‰ to 16.2‰ (Figure 4.4B), indicating a potential range of more than three trophic levels (of ~3.4‰ $\delta^{15}N$) in this benthic baseline species. The ecosystem average $\delta^{15}N$ of *P. ulvae* was 9.8±1.5‰ (Figure 4.4A). In contrast, the $\delta^{15}N$ Values of *M. edulis* were less variable, ranging from 8.6‰ to 17.2‰ (Figure 4.5B) with an average $\delta^{15}N$ value of 10.7±1.6‰ (Figure 4.5A). There were distinct visual differences between methods of baseline assessment. For the benthic baseline, medium-scale (25 km) interpolation of measured values showed relatively low heterogeneity in the baseline (Figure 4.4C, $\delta^{15}N$ between 9–11‰), whereas small-scale interpolation showed distinct 'hotspots' where baseline $\delta^{15}N$ levels were higher than 12‰ (Figure 4.4E). In contrast, both medium- and small-scale modelled baselines (Figure 4.4D and 4.4F) showed high spatial heterogeneity with $\delta^{15}N$ values ranging from 8–14 ‰. Modelled values were based upon the adjusted multiple linear regression models, $\delta^{15}N$ of the benthic *P. ulvae* was best explained by sediment grain size, sediment erosion, depth, mean salinity, distance to the nearest gulley, distance to the nearest delta and distance to the nearest source of freshwater (Table S1). However, the best model fit explained only 24% of the variation in $\delta^{15}N$ of the benthic baseline, which may explain the differences in spatial heterogeneity between interpolated measured and modelled baselines (Figure 4.4). Differences between medium- and small-scale measured pelagic baselines (Figure 4.5C and 4.5E) were remarkably low, but both baselines showed strongly elevated $\delta^{15}N$ values (>13‰) in the Eems-Dollard estuary. Although the model explained 51% of the total variation in pelagic (M. edulis) $\delta^{15}N$ values, both medium- and small scale interpolated modelled baselines failed to capture the elevated $\delta^{15}N$ values in the Eems-Dollard estuary (Figure 4.5D and 4.5F). $\delta^{15}N$ of pelagic *M. edulis* was best explained by sediment grain size, depth, mean salinity, distance to the nearest mussel bed, distance to the nearest gulley and distance to the nearest source of fresh water (Table S2).

Figure 4.3 (left): Maps of secondary consuming focal species with measured $\delta^{15}N$ signals indicated by symbol size A) *Carcinus maenas*, B) *Chelon labrosus*, C) *Balanus Crenatus*, D) *Clupea harengus*. Sample points of baseline species are indicated in green (benthic baseline, *Peringia ulvae*) and blue (pelagic baseline, *Mytilus edulis*).

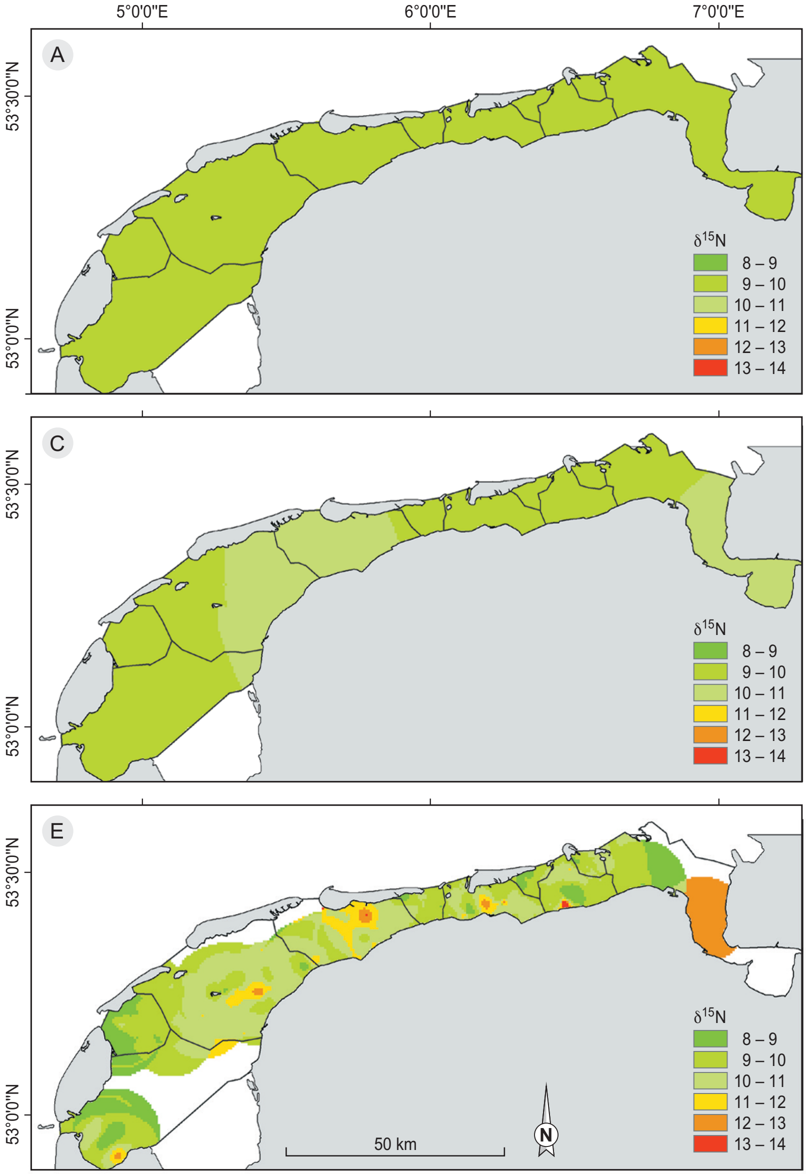
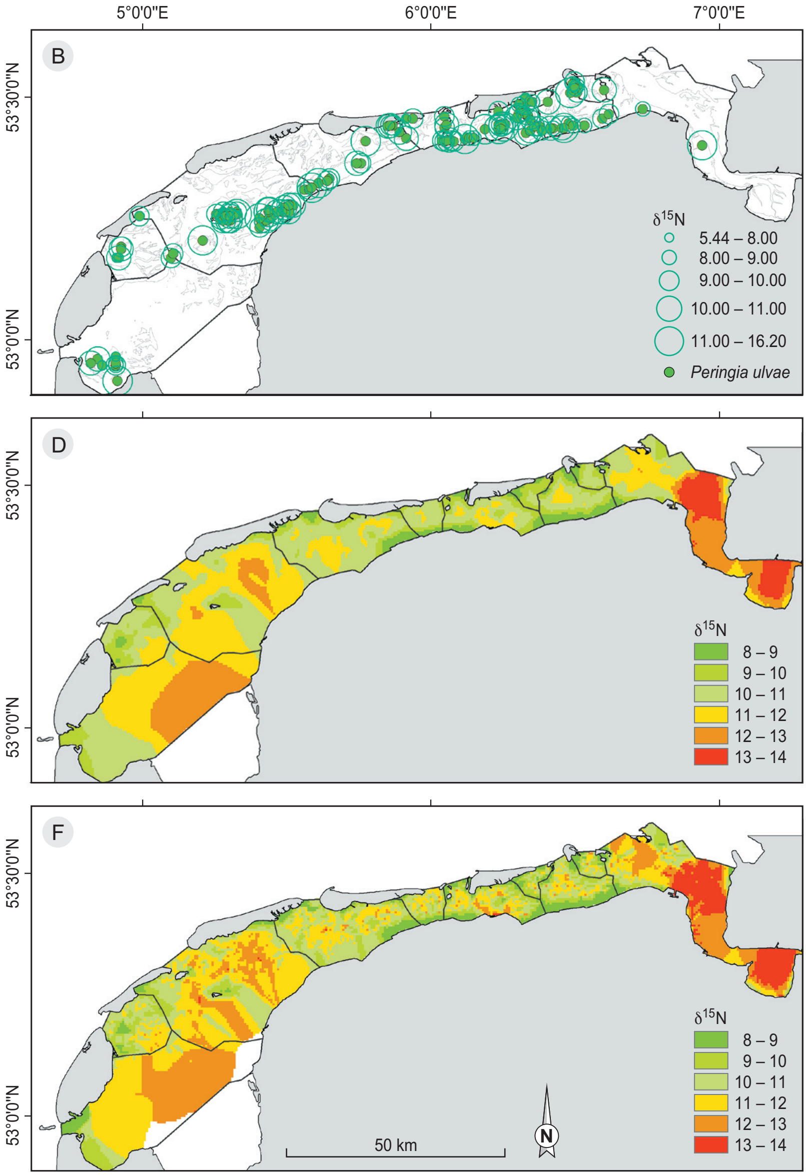


Figure 4.4: Comparison of pelagic baselines (*Peringia ulvae*, sampled on floating buoys) on various spatial scales. A) Measured large-scale spatial pattern, calculated by the mean value of all *M. edulis* sampling points, B) sampling point locations (n = 126), C) measured medium-scale interpolated spatial patterns, D) modeled medium-scale interpolated spatial patterns, E) measured small-scale interpolated spatial patterns F) modeled small-scale interpolated spatial patterns. The



colours shown in the legend belong to figures A and C-F and represent the $\delta^{15}N$ value of the baseline, where the gradient from green to red colors correspond with respectively low to high $\delta^{15}N$ values.

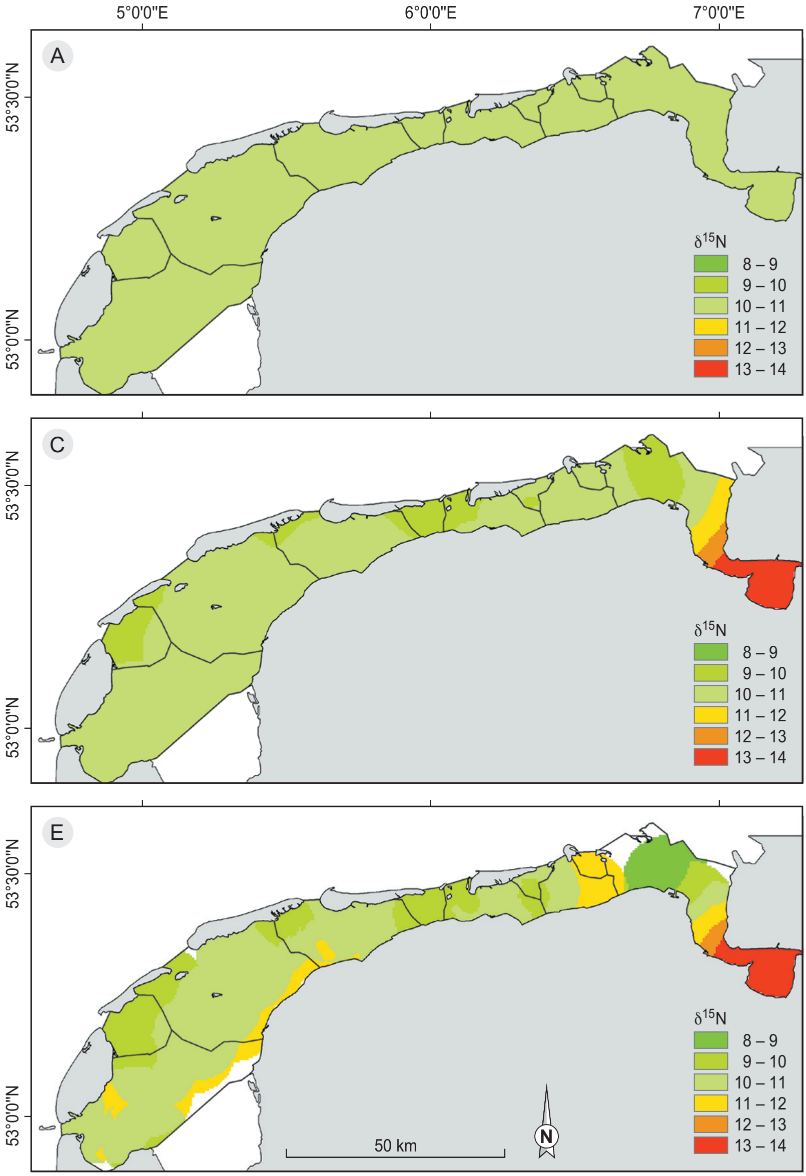
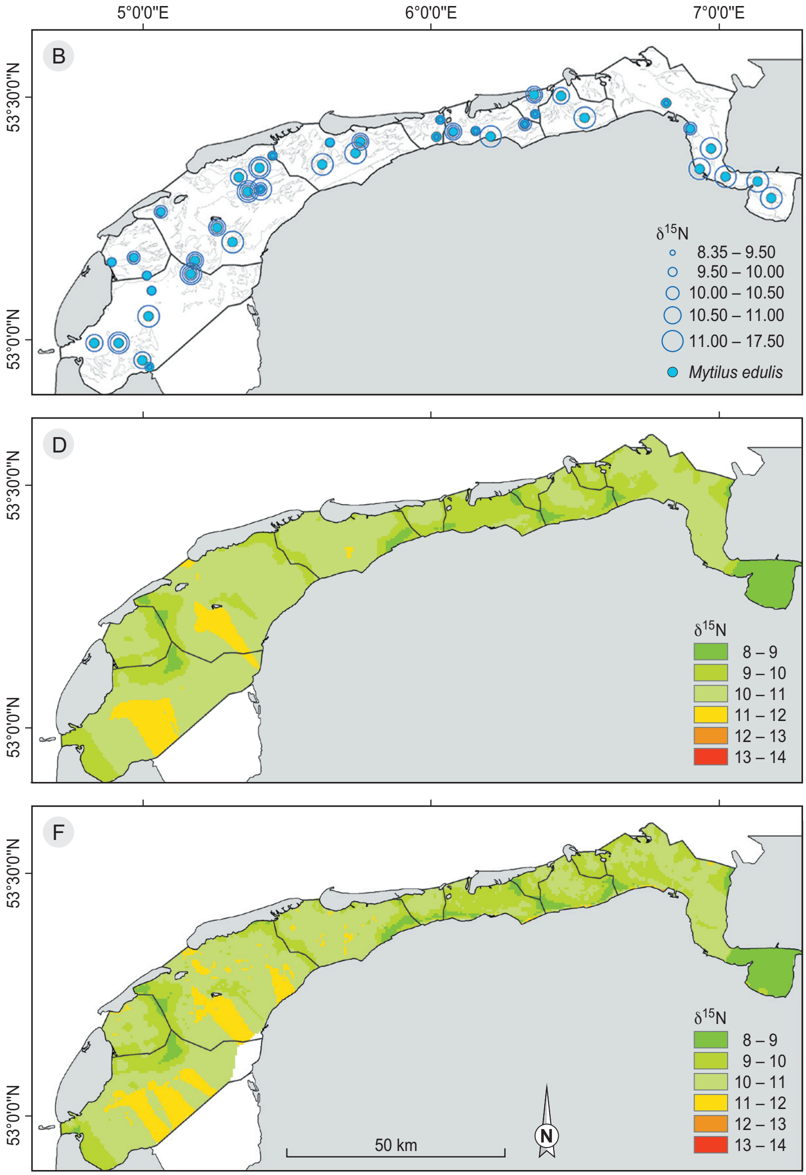


Figure 4.5: Comparison of pelagic baselines (*Mytilus edulis*, sampled on floating buoys) on various spatial scales. A) Measured large-scale spatial pattern, calculated by the mean value of all *M. edulis* sampling points, B) sampling point locations (n = 41, C) measured medium-scale spatial patterns, D) modeled medium-scale spatial patterns on the basis of environmental factors, E) measured small-scale spatial patterns F) modeled small-scale spatial patterns on the basis of envi-



ronmental factors. The colours shown in the legend belong to figures A and C-F and represent the δ¹⁵N value of the baseline, where the gradient from green to red colors correspond with respectively low to high δ¹⁵N values.

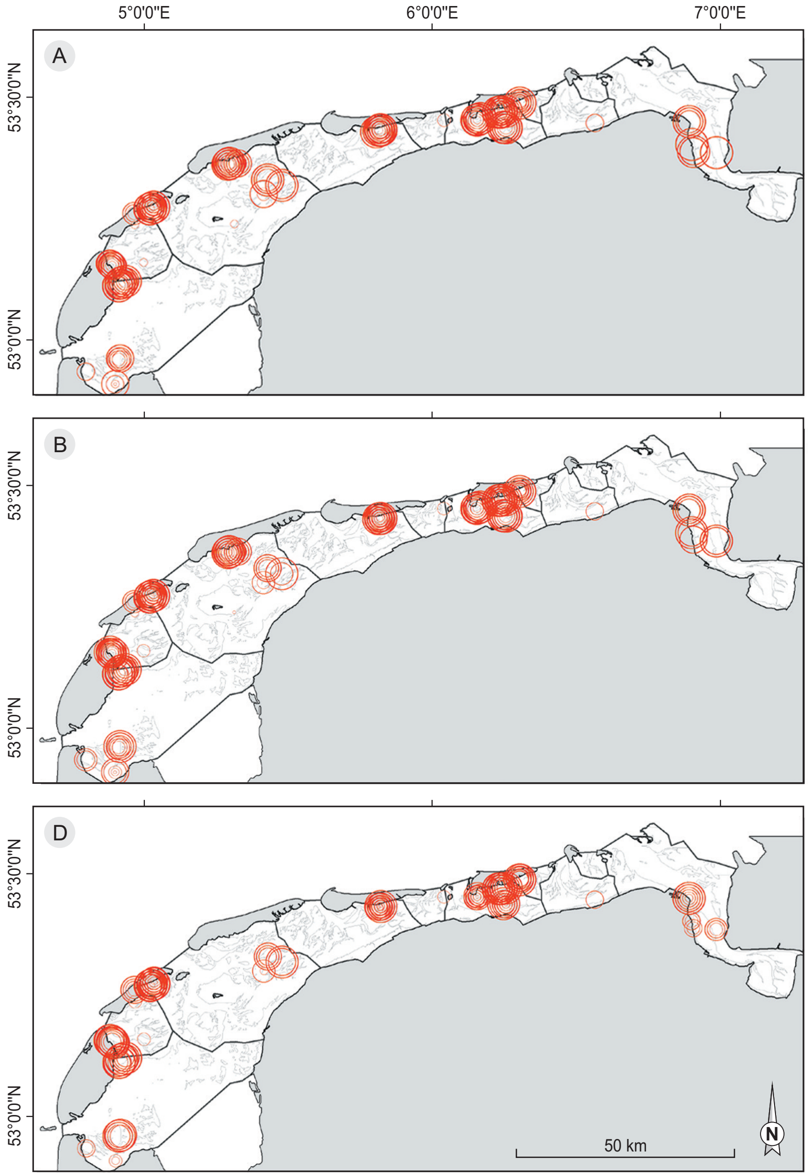
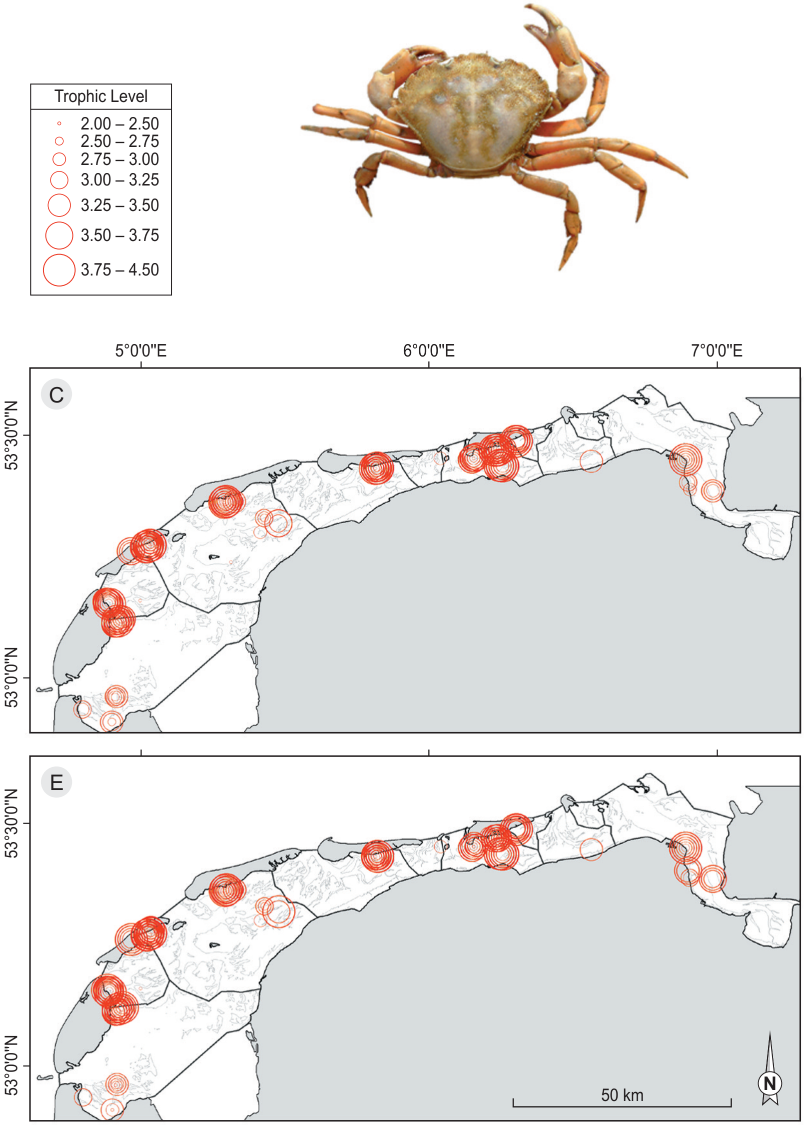
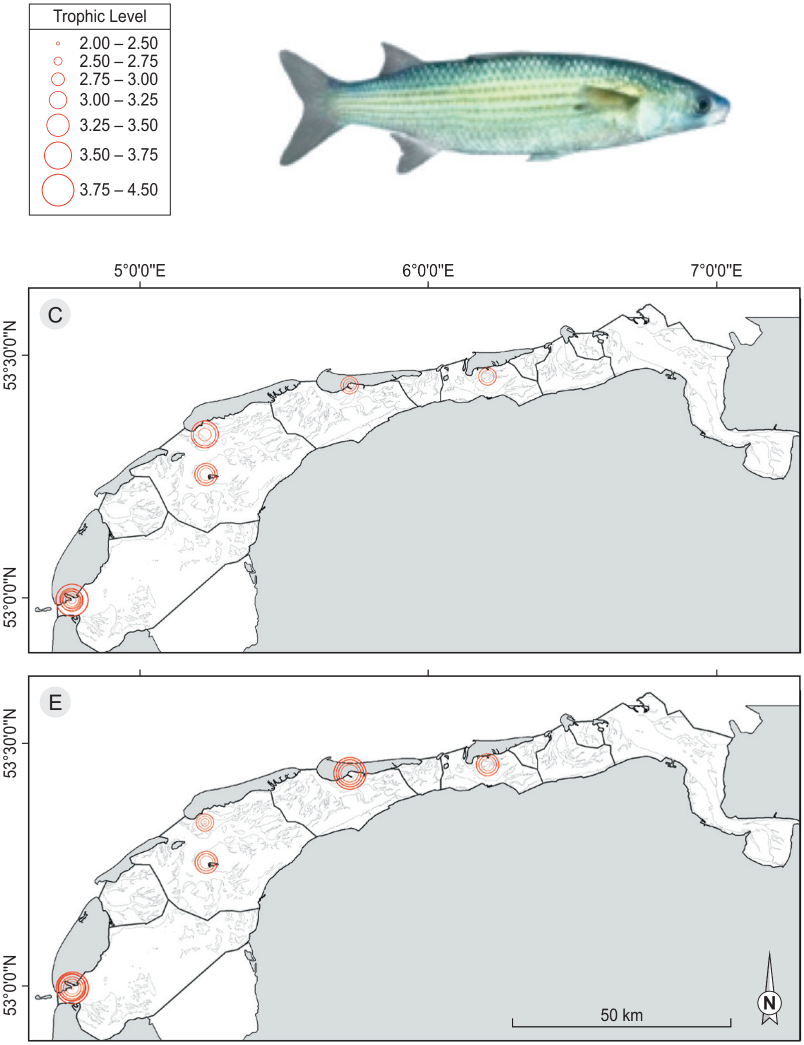


Figure 4.6: Calculated trophic levels of the benthic, low mobile (locally foraging) Shore Crab, *Carcinus maenas*, assuming the different base lines shown in figures 4.4 and 4.5. A) Measured large-scale spatial pattern, calculated by the mean value of all *C. maenas* sampling points, B)



sampling point locations (n = 126), C) measured medium-scale spatial patterns, D) modeled medium-scale spatial patterns, E) measured small-scale spatial patterns F) modeled small-scale spatial patterns.

Figure 4.7: Trophic levels of the benthic, mobile and spatially foraging Thick-lipped Mullet, *Chelon labrosus*, (n = 62) calculated by different baselines. A) Measured large-scale spatial pattern, calculated by the mean value of all *C. labrosus* sampling points, B) sampling point locations



(n = 126), C) measured medium-scale spatial patterns, D) modeled medium-scale spatial patterns, E) measured small-scale spatial patterns F) modeled small-scale spatial patterns.

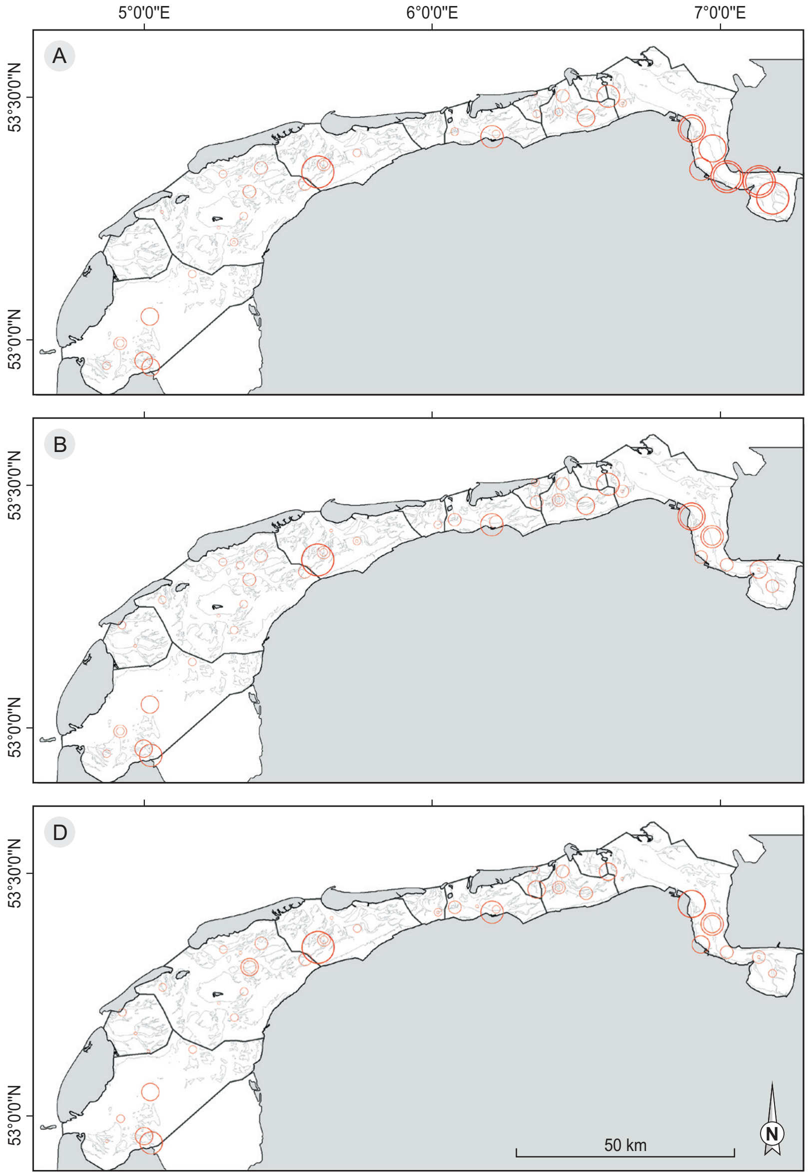
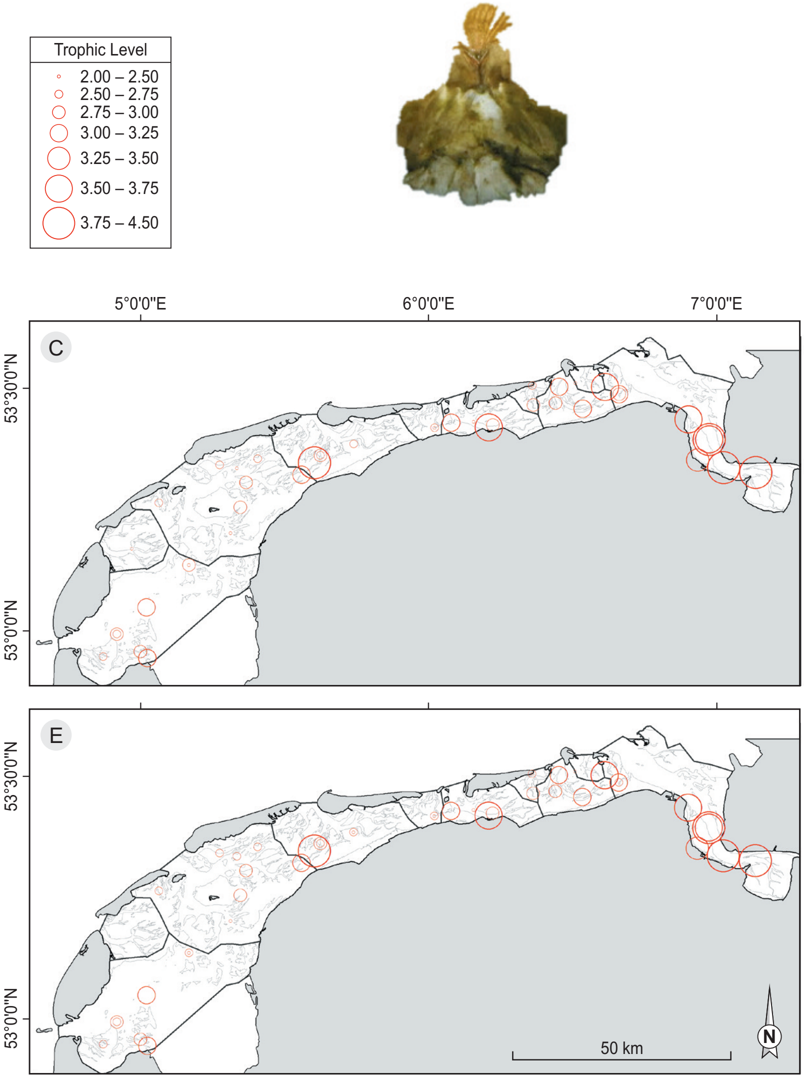
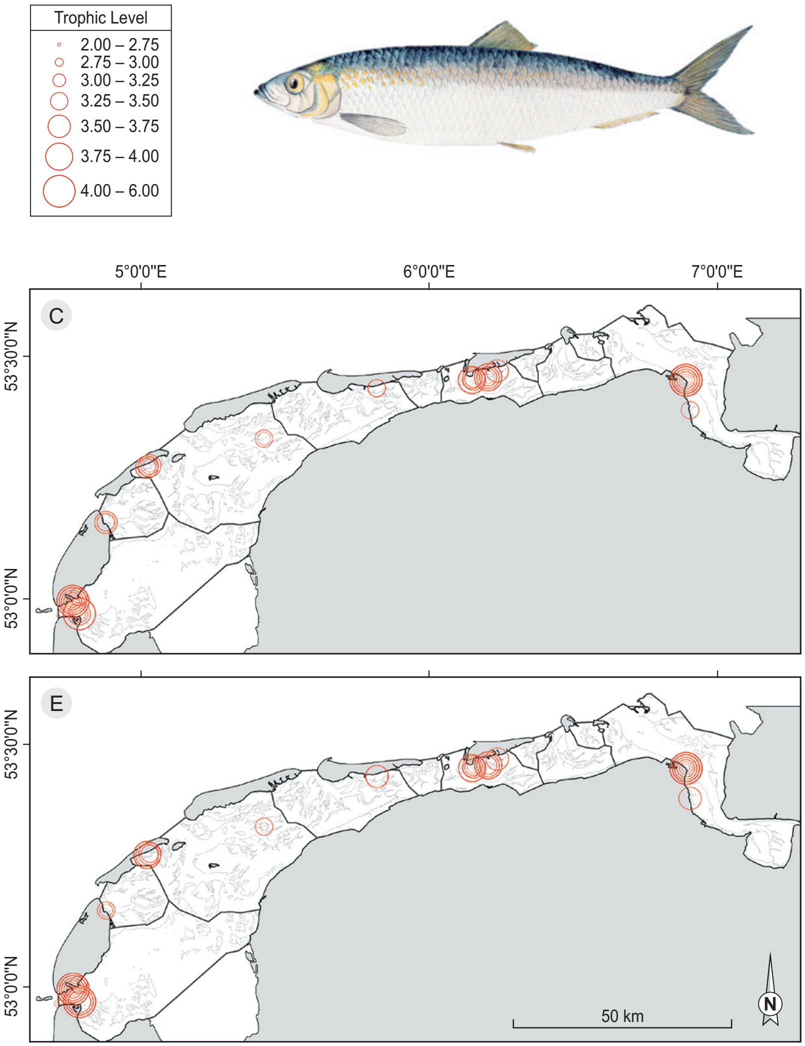


Figure 4.8: Trophic levels of the pelagic, sessile and locally foraging Barnacle, *Balanus crenatus*, calculated by different baselines. A) Measured large-scale spatial pattern, calculated by the mean value of all *B. crenatus* sampling points, B) sampling point locations (n = 41), C) measured



medium-scale spatial patterns, D) modeled medium-scale spatial patterns, E) measured small-scale spatial patterns F) modeled small-scale spatial patterns.

Figure 4.9: Trophic levels of the pelagic, mobile and spatially foraging Herring , *Clupea harengus*, calculated by different baselines. A) Measured large-scale spatial pattern, calculated by the mean value of all *B. crenatus* sampling points, B) sampling point locations (n = 41), C) measured



medium-scale spatial patterns, D) modeled medium-scale spatial patterns, E) measured small-scale spatial patterns F) modeled small-scale spatial patterns.

We finally assessed the effects of using spatially explicit baselines for determining trophic levels of higher consumers using the four model species: *C. maenas*, *C. labrosus*, *B. crenatus* and *C. harengus*. Shore crab, *C. maenas*, showed high within-site variation (TL 2.50–4.50) and some between-site variation (TL 2.75–3.75), but spatial heterogeneity did not distinctly differ between baseline methods (Figure 4.6). Similarly, *C. labrosus* showed some within-site variation (TL 2.75–3.75) and between-site variation (TL 3.25–3.75), but the use of different baselines did not affect trophic levels of this species (Figure 4.7). Thus, for these species (*C. maenas* and *C. labrosus*), spatial heterogeneity of the baseline was not relevant for trophic level calculations.

In contrast, the pelagic consumer *C. crenatus* (barnacle) showed no within-site variation, but high between-site variation (TL 2.50–4.50) and this spatial variation was highly affected by the use of different spatially explicit baselines (Figure 4.8). Especially in the Eems-Dollard estuary, where elevated baseline $\delta^{15}N$ levels were measured (Figure 4.5C and 4.5E), trophic levels of *C. crenatus* were overestimated in this area when not using a spatially explicit baseline (Figure 4.8A) or modelled spatially explicit baselines (Figure 4.8D and 4.8E). Finally, we also determined trophic levels of *C. harengus* using these methods and show that within-site variation (TL 2.00–6.00) as well as between site variation (TL 3.25–6.00) were high (Figure 4.9). However, calculated trophic levels of *C. harengus* did not very much differ between baseline methods (Figure 4.9A-E) and this mobile pelagic species thus seems insensitive to spatial heterogeneity of the baseline.

Discussion

We found that the inferred spatial heterogeneity of baselines (benthic and pelagic) strongly depended on the method used to determine spatial heterogeneity: (medium- and small-scale) modelled baselines indicated too much spatial heterogeneity due to insufficient model estimates, whereas interpolations (medium- and small-scale) of measured data exposed important spatial heterogeneity, especially in the Eems-Dollard estuary for the pelagic baseline. This suggest that ecosystem-wide averages for assumed food web baseline values would not be appropriate in this case for both groups of consumers. Also, we found that the trophic level estimates of the four model consumers with different mobility (low vs. high mobility) and feeding mode (dominantly pelagic vs. benthic) dependent strongly on how their food base line was measured. The benthic shore crab, *C. maenas*, with low mobility, the benthic thick-lipped mullet, *C. labrosus* with higher mobility and the mobile, pelagic feeding Atlantic herring, *C. harengus* were relatively insensitive to baseline heterogeneity as determined by the different methods. In contrast, the sessile pelagic barnacle *B. crenatus* did show strong sensitivity to spatial baseline heterogeneity of medium- and small scale interpolated measured baseline values, especially in the Eems-Dollard estuary. These results suggest food web studies need to take different base lines assumptions into account for different species. For highly mobile species that integrate baseline values from across landscape gradients, baseline heterogeneity does not need to be taken into account because within site variation of these species can be higher than between-site variation due to for instance diet type (generalist) or mobility. For such species, the use of an integrated baseline (averaged value across the study system) may not lead to over- or underestimates of trophic levels across a landscape. However, some sessile species, such as our model species C. crenatus, may be much more sensitive to baseline spatial heterogeneity and the use of one integrated baseline value may lead to incorrect estimates of trophic levels of such species. Thus, the use of spatially explicit baselines may only be needed for calculating trophic levels of sessile specialists, whereas for mobile species or generalist feeders, spatial heterogeneity of $\delta^{15}N$ baselines may have less effect on trophic level calculation than e.g. species size or diet type in a highly-connected coastal ecosystem.

Spatial heterogeneity in $\delta^{15}N$ was only partly explained (24 and 51 % for benthic and pelagic baseline respectively) explained by abiotic conditions in our models (sediment grain size, sediment erosion, orbital velocity, exposure time, salinity in rain season, mean salinity, depth, distance to the nearest mussel bed, distance to the nearest gully, distance to the nearest delta and the distance to the nearest source of freshwater). This indicates that we may have missed still some relevant factors that contribute to variation in $\delta^{15}N$ values of primary consumers. Next to the factors that we have taken into account, potential factors that affect s $\delta^{15}N$ values are nutrient availability, nitrogen source, nitrogen fixation, decomposition rates, etc. (Hanson, Jones & Harris 2018; Barnes et al. 2009; Jennings & Warr 2003; Mullin et al. 1984; Peterson & Fry 1987). In addition, biotic factors such as consumers' size, age and type of tissue used for analysis may also contribute to (variation in) $\delta^{15}N$ values of a primary consumer (Minagawa & Wada 1984, Post 2002, Hahn, Hoye, Korthals et al. 2012). Our analyses indicated that the most prominent baseline heterogeneity was observed in the Eems-Dollard estuary, that part of the ecosystem that is most strongly affected by riverine freshwater and organic matter input. Elevated baseline $\delta^{15}N$ values in this estuary may be caused by a different nitrogen source in this estuary (Helder et al. 1983, Hahn et al. 2012) or due to a stronger impact of nitrifying bacteria throughout the salinity gradient of this estuary (Helder & de Vries 1983). Similar patterns have been observed in other estuaries that are also known sources of nutrients to adjacent coastal systems (e.g. Baird & Ulanowicz 1993, Vitousek et al. 1997).

In addition to the spatial heterogeneity in $\delta^{15}N$ values of consumers, for three out of the four model species (shore crab, mullet, herring) we found high within-site variation of $\delta^{15}N$. Sensitivity to baseline heterogeneity of these species was also low due to this high within-site variation (Figure S1). Within-site heterogeneity implied variation of up to two trophic levels in shore crab. This high within-site variation of consumer $\delta^{15}N$ may be explained by a range of factors such as sample size, animal size, mobility, diet choice, etc. (Minagawa & Wada 1984, Hobson, Schell, Renouf et al. 1996, Post 2002, Cronin et al. 2015; Lehmitz & Maraun 2016). In shore crab (*C. maenas*), some within-site variation of $\delta^{15}N$ may be explained body size (Figure S1A+B), and individual variation in diets. Shore crabs are generalists eating molluscs, mainly bivalves, crustaceans, polychaetes, green algae and seagrass seeds (Crothers 1967, Grosholz & Ruiz 1996, Infantes et al. 2016). Individuals may however specialize in one type of prey, and prey-type may also differ among size classes (Crothers 1967) Although we have excluded all shore crabs smaller than 1.5 cm from our analyses, the observed within-site variation in shore crab $\delta^{15}N$ can thus be attributed to both the range of prey types consumed by crabs and individual differences in feeding behaviour among crabs. Hence, spatial baseline heterogeneity does not seem to matter for a generalist such as shore crab due to large within-site differences. Thick-lipped grey mullet (*C. labrosus*) displays some within-site variation (Figure S1D) and is considered to be a stronger specialist than *C. maenas*. Mullet is a schooling fish species that feeds on plankton, benthic bacterial and diatoms and macroalgae. As this species is known to display high site fidelity (Green et al. 2012), the $\delta^{15}N$ signal of these food sources may be easily integrated, leading to low within-site variability in $\delta^{15}N$. However, this may also be due to the small sampling size a mullet on each site. Within-site variation may also be to some extent explained by variation in length of fish caught on each sampling site as mullet displayed higher d15N with increasing length (Figure S1C). The sessile barnacle (*B. crenatus*) showed little within-site variation. This may be due to the immobility of the species that feeds on zooplankton, and larger phytoplankton and suspended organic matter that is passing by (Grosberg 1982), strongly integrating over temporal heterogeneity. Lastly, within-site variation in herring, *C. harengus*, may also be partly due to variation in size classes per site (Figure S1E). Prey type may differ throughout fish ontogeny and herring larvae are known to primarily feed on phyto- and zooplankton (Green et al. 2012), whereas they consume larger organisms of probably higher trophic levels, such as fish larvae, copepods and other crustaceans when they mature (Whitehead et al. 1988). In addition, herring can be highly mobile, and individuals present within one site may have integrated prey $\delta^{15}N$ signal from across a large area.

We showed that differentiation between benthic and pelagic baselines may be important for trophic level estimates. In our study system, the benthic baseline was on average 8.2‰ ($\delta^{15}N$) lower than the pelagic baseline. As these mean baseline values differed significantly (Figure S2), we took feeding mode – benthic / pelagic – into account for correct baseline construction, in line with previous studies (Post 2002, Chouvelon et al. 2012). This difference in $\delta^{15}N$ between pelagic and benthic baselines is probably due to differences in nitrogen sources between benthic and pelagic primary producers (Chouvelon et al. 2012, Sherwood & Rose 2005). For instance, benthic microbial films may consist of algal and bacterial communities.

We assessed whether mobility of a consumer affects its sensitivity to baseline spatial heterogeneity and we found, in line with our hypothesis, that mobile species, that integrate baseline values across landscape gradients, indeed were less sensitive to spatial heterogeneity of the baseline. Contrastingly, sessile specialists, such as our model species *B. crenatus*, appeared to be extremely sensitive to baseline heterogeneity when calculating trophic levels of these species.

Overall we conclude that taking spatial baseline heterogeneity (interpolation of measured baseline values) into account is essential for sessile specialist consumer species, especially across landscape gradients such as estuaries. Estuaries may provide an alternative nitrogen source to a coastal ecosystem that may lead to baseline heterogeneity that in turn affects trophic level estimates of sessile specialists. For mobile or generalist consumer species, spatial baseline heterogeneity seemed to have very limited impact on trophic level estimates, as these species are strongly spatially averaging over all kinds of isotopic signals. However, we have only assessed this for four model species and we thus recommend careful consideration of the impact baseline heterogeneity for each individual species.

Coastal ecosystems are rapidly degrading on a global scale. (Jackson 2001, Lotze et al. 2005, 2006). To conserve these valuable ecosystems, various ecological indicators are used to assess ecosystem status (Christianen et al. 2017b and references therein). The Marine Strategy Framework Directive (MSFD, Rombouts et al 2013) as well as in other directives now include Shannon-Wiener diversity and species indices, but less often indicators for changes in the food web structure of ecosystems are used (but see Shannon et al 2009, 2014). This is in contract with the progress made in the key role of trophic complexity in the functioning of ecosystem (Duffy et al 2007). This may generate a potential mismatch between conservation goals and the indicators that are used to measure policy success. Alternative food web indicators such as trophic levels of consumers (TL) may provide additional insights in ecosystem functioning of target conservation areas such as the Wadden Sea World Heritage Site (Christianen et al. 2017b). For instance, trophic levels estimates enable cross-system comparisons (e.g., Olff et al 2009) and are already used to assess impacts of fisheries across marine ecosystems (Vizzini et al 2009, Shannon et al. 2014). In addition, estimates of top-down versus bottom-up forces in coastal food webs (Frank et al 2007) may have different conservation implications. We hope that our study contributes to the accurate use of trophic level estimates as food web indicators for the assessment of ecosystem functioning, which can be used to improve conservation of rapidly degrading coastal ecosystems.

Acknowledgements

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Supplemental information

Table S1: Multiple linear regression table for *Peringia ulvae* of dependence of δ^{15}N level on environmental conditions.

adjusted model *P. ulvae*	R^2	B	SE B	Error	P	significance
(Intercept)	0.236	8.08E+00	4.71E+00	1.717	0.090227	.
sediment_grain size		8.72E-03	5.89E-03	1.482	0.142739	ns
sediment_erosion		−6.02E-06	1.43E-04	−0.042	0.966523	ns
depth		−1.83E-02	5.08E-03	−3.596	0.000584	***
salinity_mean		−5.14E-02	1.42E-01	−0.361	0.718944	ns
distance_to gulley		−3.16E-01	1.82E-01	−1.738	0.086441	.
distance_to delta		1.47E-01	6.42E-02	2.293	0.024712	*
distance_to freshwater		2.82E-02	1.47E-02	1.914	0.059568	.

Table S2: Multiple linear regression table *Mytilus edulis*.

adjusted model *M. edulis*	R^2	B	SE B	Error	P	significance
(Intercept)	0.511	7.3704147	1.5755860	4.678	2.10E-05	***
sediment_grain size		0.0019988	0.0034197	−0.584	0.561411	ns
Depth		0.0013079	0.0004647	2.814	0.006886	**
salinity_mean		0.1649801	0.0590587	2.793	0.007282	**
Distance to_musselbed		0.1182359	0.0436609	2.708	0.009139	**
distance_to gulley		0.6145629	0.1098075	−5.597	8.28E-07	***
distance_to freshwater		0.0229197	0.0055126	−4.158	0.000121	***

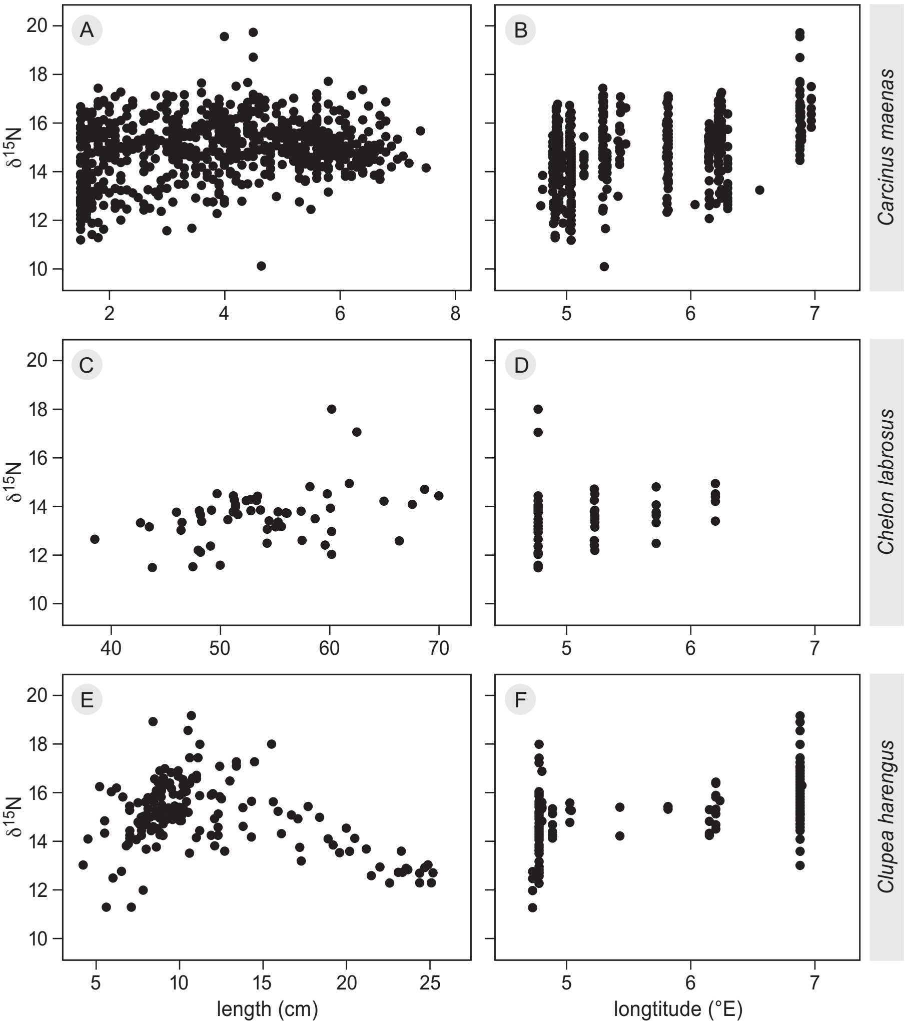

Figure S.1: Relationship between A) *C. maenas* length and $\delta^{15}N$, B) *C. maenas* sampling location expressed by longtitude and $\delta^{15}N$, C) *C. labrosus* length and $\delta^{15}N$, D) *C. labrosus* sampling location expressed by longtitude and $\delta^{15}N$, E) *C. harengus* length and $\delta^{15}N$, F) *C. harengus* sampling location expressed by longtitude and $\delta^{15}N$.

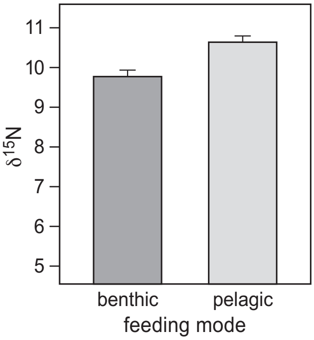

Figure S.2: Comparison of averages of benthic (*Peringia ulvae*) and pelagic (*Mytilus edulis*) feeding mode baslines. n = 127 for benthic and n = 110 for pelagic baselines. Error bars represents standard error of mean (SEM). Unpaired t-test T = 4.186, df = 235, $P < 0.001$.

CHAPTER 5

Unexpected diet preferences of Eurasian Spoonbills in the Dutch Wadden Sea: Spoonbills mainly feed on small fish instead of shrimp

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Abstract

After an historical absence, over the last decades Eurasian spoonbills *Platalea leucorodia* leucorodia have returned to breed on the barrier islands of the Wadden Sea. The area offers an abundance of predator-free nesting habitat, low degrees of disturbance, and a spatially seemingly unlimited intertidal feeding area with increasing stocks of brown shrimp *Crangon crangon*, their assumed main prey. Nevertheless, newly established and expanding colonies have surprisingly quickly reached plateau levels. Here we verified the often stated assertion that spoonbills mainly rely on brown shrimp as food, by quantifying the diet of chicks on the basis of regurgitates and by analyses of blood-isotopes using stable isotope Bayesian mixing-models. Both methods showed that, rather than brown shrimp being the staple food of spoonbill chicks, small flatfish (especially plaice *Pleuronectes platessa*) and gobies (*Pomatoschistus* spp.) were their main prey. Unlike shrimp, small flatfish have been reported to be rather scarce in the Wadden Sea in recent years, which may explain the rapid saturation of colony size due to food-related density-dependent recruitment declines of growing colonies. By way of their diet and colony growth characteristics, spoonbills may thus indicate the availability of small fish in the Wadden Sea. We predict that the recovery of former densities of young flatfish and other juvenile/small fish in the Wadden Sea would be tracked by changing diets (more fish) and an increasing size of the colonies across the Wadden Sea.

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Introduction

The Wadden Sea, the area of shallows and intertidal flats between the European mainland and the barrier islands which are bordered to the North Sea, provides vast habitat for marine and estuarine species, including those that connect the Wadden Sea with ecosystems elsewhere on the globe, i.e. the migratory shorebirds (Swennen, 1976; van de Kam et al., 2004; Reise et al., 2010; van Roomen et al., 2012). This ecosystem has been subject to many external forces, many of the human ones contributing to the degradation of ecosystem functioning (de Jonge et al., 1993; Wolff, 2005; Eriksson et al., 2010). In recent decades, in temporal association with policy recognitions such as RAMSAR status and, more recently, as a UNESCO World Heritage Site, attempts have been made to conserve and restore the biodiversity and ecosystem functioning of the Wadden Sea (Boere and Piersma, 2012).

The return of Eurasian spoonbills *Platalea leucorodia leucorodia* as a breeding bird of the Wadden Sea barrier islands, probably after an historical absence of many centuries due to human persecution (de Goeij et al., 2015), counts as a tangible result of successful conservation measures. From the late 1960s onwards, the number of spoonbill breeding pairs increased exponentially in the Dutch Wadden Sea (de Goeij et al., 1985; Lok et al., 2009; Oudman et al., 2017), caused by profitable circumstances on the Wadden Sea islands due to enforced protection of foraging and breeding areas (de Goeij et al., 1985; Kemper, 1986a; van der Hut, 1992). This increase in numbers has been encouraged by resettlement from mainland colonies threatened by red fox predation, and from other forms of immigration, but has also been due to local recruitment (Lok et al., 2009). Indeed, the Wadden Sea seems to provide all what reproductively active spoonbills need: plenty of suitable nesting places with little or no predation, very low degrees of disturbance, and seemingly unlimited extents of foraging area in the form of shallow gullies and tidal flats.

It has therefore been surprising that newly established and expanding colonies in the Wadden Sea quickly reached plateau levels (Lok et al., 2009; Oudman et al., 2017), the increase of the total breeding numbers being driven to a large extent by the formation of new colonies near previously unoccupied areas of intertidal flat. Growing colonies show signs of density dependence as (1) the number of fledglings per nest declined with colony size (Lok et al., 2009; Oudman et al., 2017) and (2) the post-fledging survival rates of spoonbills declined with an increase of overall population size (Lok et al., 2013). In view of the large unused extents of what appears high quality breeding habitat, it has been suggested that food might be the factor causing density dependence and limiting population size (Oudman et al., 2017).

Shrimp *Crangon crangon* have been repeatedly reported as being the main prey of spoonbills, especially during the chick-rearing period (Tinbergen, 1933; Kemper, 1986a,b; Wintermans and Wymenga, 1996; Altenburg and Wymenga, 1997; Figure

5.1). Only, de Goeij et al. (1985) indicated that a high availablity of young plaice in pools in the Wadden Sea during low tide would provide easy prey for spoonbills. That food of spoonbills might be limiting, and that several colonies have reached plateau levels more than ten years ago (e.g. Terschelling and Schiermonnikoog), can possibly be explained either by (1) shrimp becoming more abundant (Tulp et al. 2012) but shrimp not actually being the staple food, or (2) by shrimp availability actually being smaller than thought, e.g. by high fishing pressure as reported by Tulp et al. (2016). In this study we aim to examine these possibilities by a study of the diet of nestling spoonbills, across the colonies in the Dutch Wadden Sea, using both regurgitates and isotopic Bayesian mixing-models (SIAR) based on stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$) in bird blood and prey tissue samples to estimate the diet of chicks. SIAR was used to model the isotopic (food web) position of spoonbills relative to their prey, here, by using stable carbon and nitrogen isotopes; $\delta^{13}C$ is useful to discriminate between marine and terrestrial organisms while $\delta^{15}N$ is useful in studying

Figure 5.1: A spoonbill chick producing a regurgitate upon being held after capture. At first sight such regurgitates only show the remains of shrimp.

trophic position (Hobson & Welch 1992, Polis & Hurd 1995, Post 2002). Knowing the staple foods of spoonbills we suggest the best management practices for the Wadden Sea serving conservation goals.

Material and Methods

Spoonbills are tactile foragers and have altricial chicks that are fed by their parents (Hancock et al., 2010). After a breeding period of about 25 days, during which both parents incubate the eggs, most chicks hatch from early to mid-May (Lok et al., 2017). During the breeding season spoonbills are bound to the nest and therefore restricted to a foraging range of less than 30–40 km from the breeding colony (Altenburg et al. 1997). Although this foraging range allows them to forage in both marine and freshwater resources, chicks appear mainly fed with marine prey likely captured in the Wadden Sea (El-Hacen et al., 2014).

The diet of Spoonbill chicks, in colonies on the barrier islands of the Wadden Sea, was assessed from regurgitates and from blood stable isotope analyses. Whereas regurgitates reflect the diet on the day of collection, stable isotope analyses indicate the diet integrated over longer periods: a few days if based on blood plasma, a few weeks if based on red blood cells (e.g. Dietz et al., 2010; Hahn et al., 2012). Both methods are ideal for diet reconstruction, with the caveat that small prey and easily digestible prey may be missed when using diet reconstruction based on regurgitates, while the isotopic mixing model SIAR is an indirect method and requires precise assumptions (e.g. about discrimination factors and selection of potential prey for SIAR).

From 17 May – 12 July 2012 and from 12 June – 7 August 2013, a total of 301 chicks aged 15-35 days were examined in colonies on five islands in the Dutch Wadden Sea (Figure 5.2). Within 1 h after capture the birds were colour-ringed, body size measures were taken (see Lok et al., 2014) and a blood sample of 150–400 μl was taken from the brachial vein in heparinized capillaries. Within 3 hrs after sampling, blood plasma and red blood cells (RBC) were separated in Eppendorf cups in a haematocrit centrifuge (microfuge Sigma 1–13, 6 min on 5000 rpm). Plasma and RBC were pipetted in separate glass vials. Samples were transported in a bag with cooling elements for maximally 4 hrs before storage at –20°C until analysis. To obtain the isotope values of potential prey, 209 food items were collected between 12 April – 17 May 2012 and from 2 May – 12 July 2013 in all potential feeding habitats of spoonbills. Prey collection occurred on locations where spoonbills were foraging on that moment or at least were known to forage frequently. The proportions of prey species as calculated with regurgitate analyses was used as a prior for prey selected in the stable isotopic based diet reconstruction SIAR (all prey species that compromised >2% of

Figure 5.2: Map with overview of the Spoonbill colonies on the five Dutch Wadden Sea islands where Spoonbill samples were collected.

the total diet). In order to limit the number of prey input in SIAR, we combined all important prey species in freshwater and mixture sources, while using all important prey of the marine water source. Prey categorized under 'mixture' is prey that occurs in marine, brackish and freshwater habitat types. An overview of the prey species used for diet reconstruction with help of isotopic mixing-models (SIAR) is given in Table 5.1. Carbon isotopes of prey were normalized a posteriori for the effect of lipid concentration, using a correction based on the C:N ratio given by Post et al. (2007) (Table 5.1).

Stable isotope values of spoonbill chick are shown in Table 5.2. As explained by Cherel et al. (2005) lipids extraction of plasma is required for measuring adequate

Table 5.1: Mean stable isotope values of prey used as an input for diet reconstruction in the stable isotope mixing model SIAR. Selected prey species contributed >2% of the diet assessed with regurgitate analyses. Carbon isotopes were normalized for the effect of lipid concentration ($\delta^{13}C_{lipid\text{-}corr}$), using a correction based on the C:N ratio given by Post et al. (2007).

			$\delta^{15}N$		$\delta^{13}C$		N	C:N		$\delta^{13}C_{lipid\text{-}corr}$	
Water type	**Island**	**Species**	Mean	SE	Mean	SE		Ratio	SE	Mean	SE
Marine	Texel	*Crangon crangon*	13.2	0.4	−14.9	0.6	20	3.6	0.06	−14.6	0.6
		Pleuronectes platessa	14.0	0.2	−16.2	0.5	19	3.4	0.04	−16.1	0.5
		Pomatoschistus microps	15.2	0.5	−14.3	0.2	2	3.6	0.08	−14.0	0.3
	Vlieland	*Crangon crangon*	12.2	0.2	−13.2	0.2	7	3.7	0.06	−12.8	0.2
		Pleuronectes platessa	11.6	0.3	−14.5	0.3	4	3.5	0.06	−14.4	0.3
		Pomatoschistus microps	14.1	0.6	−15.4	0.8	3	4.0	0.06	−15.1	1.3
	Terschelling	*Crangon crangon*	12.9	0.2	−14.4	0.1	17	3.7	0.03	−14.1	0.1
		Pleuronectes platessa	12.3	0.2	−15.8	0.2	8	3.7	0.03	−15.5	0.2
		Pomatoschistus microps	14.9	0.2	−15.8	0.5	7	4.1	0.08	−15.0	0.5
	Ameland*	*Crangon crangon*	12.8	0.2	−14.6	0.3	56	3.7	0.02	−14.3	0.3
		Pleuronectes platessa	13.0	0.2	−16.4	0.4	38	3.5	0.03	−16.2	0.4
		Pomatoschistus microps	14.7	0.2	−15.5	0.4	12	4.0	0.07	−14.8	0.3
	Schiermonnikoog	*Crangon crangon*	12.3	0.2	−15.1	0.8	11	3.7	0.02	−14.8	0.8
		Pleuronectes platessa	11.8	0.4	−19.7	1.4	5	3.7	0.06	−19.3	1.4
		Pomatoschistus microps	14.7	0.2	−15.5	0.4	12	4.0	0.07	−14.8	0.3
Mixture	All islands	Total**	12.8	0.5	−24.2	0.8	44	4.3	0.20	−23.2	0.8
		Gasterosteus aculeatus (90.8%)	12.4	0.5	−24.9	0.8	40	4.4	2.20	−23.8	0.9
		Osmerus eperlanus (9.2%)	16.3	0.3	−17.1	0.2	4	3.2	0.02	−17.2	0.3
Freshwater	All islands	Total**	15.9	0.8	−27.5	0.4	46	3.4	0.06	−27.5	0.3
		Perca fluviatilis (24.1%)	18.0	0.2	−26.7	0.2	7	3.2	0.01	−26.9	0.2
		Pungitius pungitius (19.4%)	7.8	0.4	−30.2	0.5	24	3.9	0.07	−29.7	0.4
		Rutilus rutilus (56.5%)	17.8	0.3	−26.8	0.4	15	3.2	0.02	−26.9	0.4

* Because low numbers of main prey species were collected on Ameland, mean values of all islands were used.

** Dietary ratios of the main prey based on the regurgitate analysis (>2%, see table 5.2) were used to calculate the mean stable isotope values of 'mixture' and 'freshwater' for the prey input in SIAR.

Table 5.2: Mean stable isotope values of Spoonbill chicks used as an input for diet reconstruction with help of stable isotope analysis (SIAR).

Island	Tissue	$\delta^{15}N$ Mean (SE)	$\delta^{13}C$ Mean (SE)	TOC Mean (SE)	N Mean (SE)	C:N Mean (SE)
Texel (n = 48)	cells	14.82 (0.14)	−16.31 (0.23)	47.86 (0.19)	14.96 (0.07)	3.20 (0.01)
	plasma	16.41 (0.15)	−16.53 (0.20)	42.66 (0.27)	9.99 (0.10)	4.28 (0.04)
Vlieland (n = 64)	cells	15.17 (0.06)	−16.21 (0.23)	48.69 (0.14)	14.96 (0.06)	3.26 (0.01)
	plasma	16.59 (0.14)	−16.91 (0.30)	42.03 (0.26)	9.86 (0.09)	4.27 (0.03)
Terschelling (n = 60)	cells	15.01 (0.09)	−19.15 (0.41)	48.72 (0.31)	15.02 (0.10)	3.24 (0.01)
	plasma	16.56 (0.12)	−19.10 (0.43)	42.72 (0.17)	9.79 (0.06)	4.37 (0.03)
Ameland (n = 45)	cells	15.31 (0.17)	−19.07 (0.45)	49.26 (0.28)	15.13 (0.07)	3.26 (0.01)
	plasma	16.75 (0.19)	−19.25 (0.41)	42.17 (0.16)	9.92 (0.06)	4.26 (0.03)
Schiermonnikoog (n = 83)	cells	15.52 (0.10)	−19.83 (0.41)	49.32 (0.14)	15.22 (0.05)	3.24 (0.00)
	plasma	17.21 (0.10)	−20.37 (0.43)	43.33 (0.20)	10.10 (0.05)	4.29 (0.02)

$\delta^{13}C$ plasma values , especially since the C:N plasma ratios of spoonbill chicks in this study are high (>4.0). Although lipid correction is needed, we were not able to repeat the analyses with lipid-extracted samples. We did not find an *a posteriori* lipid correction model to 'normalize' bird plasma for the lipid contribution.

To reconstruct diet composition with stable isotopes, we measured the carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) of blood plasma and RBC of spoonbill nestlings and of the relevant muscle tissue of prey species. All samples were freeze-dried, before grinding them with a pestle and mortar. Next, with a microbalance (Sartorius CP2P) 0.4–0.8 mg sample material was weighted and put in 5×8 mm tin capsules. The $\delta^{13}C$ and $\delta^{15}N$ isotopes values were determined by a Thermo Flash 2000 elemental analyser coupled to a Thermo Delta V isotope ratio mass spectrometer. Isotope values were calibrated to a laboratory acetanilide standard ($\delta^{13}C$ −26.1‰ and $\delta^{15}N$ 1.3‰ calibrated on NBS-22 and IAEA-N1, respectively) and corrected for blank contribution. The results are reported on a per mill scale with respect to Vienna Pee Dee Belimnite (VPDB) for $\delta^{13}C$ and to atmospheric N_2 for $\delta^{15}N$. The replicate error on the standard, acetalinide, ranged between 0.01 and 0.05, using one standard every 2.2 to 6.3 samples. The mean diets of all birds were calculated per island for the two years combined.

The relative contribution of potential prey species to the diet of spoonbill chicks was estimated using an isotopic Bayesian mixing-model programmed in the R-package SIAR v.4.2 (Parnell et al., 2010). The SIAR model requires input of at least two stable isotopes (here $\delta^{15}N$ and $\delta^{13}C$) of a consumer, its prey, and a diet-tissue differentiation factor. As prey sources we used all prey species that occurred for >2% in the spoonbill diet assessed by regurgitate analysis (Table 5.1). In order to keep the number of food sources for SIAR low (Phillips et al. 2014), prey that occurred in freshwater or in multiple water types were grouped, since (late-breeding) spoonbills mainly forage on marine Wadden Sea sources (El-Hacen et al., 2014) . We did not measure differentiation factors ourselves so we used general ones for avian plasma (δ^{15}N: 2.82 ±0.14‰ and δ^{13}C: −0.08 ±0.38‰) and avian RBC (δ^{15}N: 2.25 ±0.20‰ and δ^{13}C: −0.35 ±0‰) as presented by Caut et al. (2009).

Regurgitates (n = 128) produced during the catching and ringing sessions (Figure 5.1) were collected individually in separate plastic bags. Regurgitates were stored in a freezer (−20°C) on the same day. Single regurgitates were put on a plate for inspection and, with water added, light-weight items such as shrimp tails, uropods, heads, claws, other whole or almost intact individuals were collected first. The remaining light-weight debris was removed by placing the regurgitate in a 800 ml glass beaker filled up with water to 600 ml and mixing it with help of a magnet and magnetic stirrer until all matter was in suspension. To remove the uninformative debris, the mixture in the beaker was carefully overflown by placing the beaker under a slowly running water tap. The remaining sample was put on a glass petri dish in order to extract all identifiable parts under a binocular microscope.

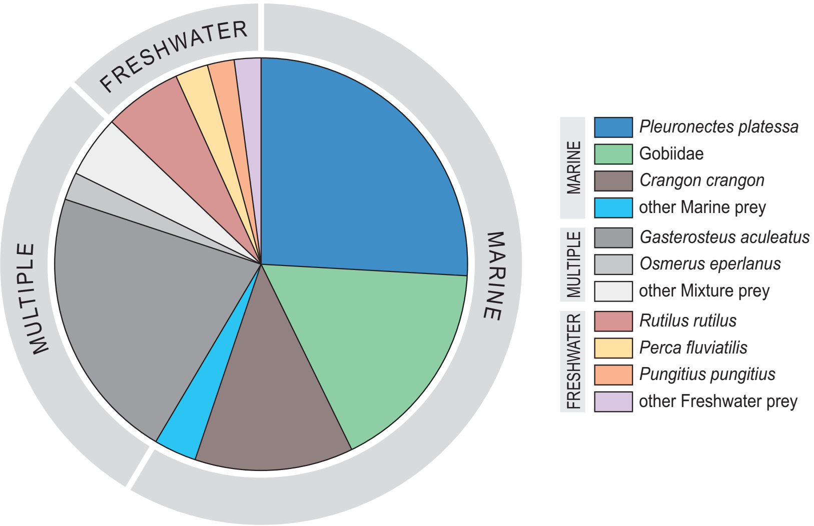


Figure 5.3: Overall composition in terms of biomass of the diet of nestling Eurasian spoonbills in the Dutch Wadden Sea based on analysis of regurgitates. Prey are divided into three water type classes; marine prey from the Wadden Sea (marine), prey that occur in more than one water type (multiple) and freshwater prey from waters from the islands or mainland (freshwater).

The items included otoliths, vertebrae, ventral and dorsal spine, cleithrums, urohyals, bullae, premaxillae, pharyngeal, dentaries, some other bones, insect fragments, crustacean fragments such as heads, carapaces, tails, telsons, uropods, (fragments of) claws (e.g. dactylus, propodus), legs, swimming pads and skin of amphibian. All parts were classified to the lowest taxonomic level possible, and the size of the parts was used to estimate the length and mass of the individuals (Leopold et al., 2001; CJC et al. unpubl. data). Note that, to calculate length and mass from the size of the parts, we made use of some regression curves developed using larger fish (Leopold et al., 2001), making the estimated length of our small fish possibly somewhat distorted. Then, we determined the number of individuals per species, accounting for size and number and orientation of parts per individual.

This study is based on samples collected in the summers of 2012 and 2013. As the sampling of different components (regurgitates, stable isotope values of prey and spoonbills) was not complete in either year, we can not compare the years and present composite values. Unless stated otherwise, notation of mean and accuracy is given by mean ±SE. Differences in diet between colonies was statistically analysed with ANOVA tests using Statistica 10 while graphs were made using Sigmaplot 12.3.

Results

The analysis of regurgitates demonstrated that nestling spoonbills on the Wadden Sea islands are fed a great variety of prey with marine and freshwater origins (Table 5.3). Summarising the information in overall mass terms (Figure 5.3), the diet of nestling spoonbills consisted for the greater part (59%) of marine prey from the Wadden Sea. Contrary to expectation, brown shrimp contributed only 12%. The main prey species were flatfish (seemingly predominantly plaice) with 26%, three-spined stickleback (22%), gobies (17%). These species had a higher biomass and length, relative to brown shrimp (*Pleuronectes platessa*: biomass 1.07 ±0.04 g, total length 36.1 ±0.5 mm (n = 1124); *Gasterosteus aculeatus*: biomass 1.40 ±0.07 g, total length 49.9 ±0.5 mm (n = 637); Gobidae: biomass 0.81 ±0.04 g, total length 39.9±0.4 mm (n = 961); *Crangon crangon*: biomass 0.19 ±0.004 1 g, total length (head-tail) 24.3 ±0.1 cm (n = 2391)).

Apart from the marine prey, the remaining part of the diet consisted of freshwater prey (29%, comprising mostly three-spined sticklebacks, Figure 5.3) and prey that could originate from more than one water type (13%). Figure 5.4 represents the diet of spoonbill nestlings (regurgitate analysis, Figure 5.4A), the diet during the previous few days (isotope analysis based on plasma tissue, Figure 5.4B), and the diet over about a month of nestling life (isotope analysis based on RBC tissue, Figure 5.4C) (Rodnan et al. 1957). Restricting the number of sources in SIAR to three (marine, mixture and freshwater) instead of five (Figure 5.4B and 5.4C), made no meaningful

Table 5.3: Spoonbill diet on the barrier islands of the Dutch Wadden Sea in 2012-2013, based on regurgitate analyses. Dietary content is expressed in percentage of the biomass contribution per prey (%). Prey that occurred <2% in the mean Wadden Sea diet (totals) were grouped.

Year: 2012–2013		**Texel** (n = 18)	**Vlieland** (n = 60)	**Terschelling** (n = 15)	**Ameland** (n = 2)	**Schiermonnikoog** (n = 33)	**Totals** (n = 128)
Habitat	**Prey item**	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
Marine	Total	34.2 (7.5)	55.8 (4.3)	71.7 (6.9)	67.7 (19.3)	69.9 (5.3)	58.5 (3.0)
	Pleuronectes platessa	20.6 (7.1)	24.4 (3.2)	33.5 (6.5)	25.6 (1.2)	28.0 (3.9)	25.9 (2.2)
	Gobiidae	5.8 (1.5)	20.6 (2.6)	17.7 (3.3)	12.9 (4.5)	16.2 (3.1)	16.9 (1.6)
	- Gobiidae	5.8 (1.5)	20.6 (2.6)	17.5 (3.2)	12.9 (5.5)	16.2 (3.1)	16.9 (1.6)
	- *Pomatoschistus minutus*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Crangon crangon	7.1 (1.2)	7.9 (1.1)	15.5 (4.5)	7.1 (4.0)	21.9 (2.1)	12.3 (1.1)
	Other marine prey	0.7 (0.3)	2.9 (1.2)	5.0 (4.1)	22.0 (17.9)	3.9 (0.7)	3.4 (0.8)
	- *Arnoglossus laterna*	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- Carcinidae	0.6 (0.3)	0.1 (0.1)	0.0 (0.0)	1.9 (0.0)	0.5 (0.5)	0.3 (0.1)
	- *Carcinus maenas*	0.0 (0.0)	0.4 (0.2)	0.6 (0.4)	0.0 (0.0)	2.0 (0.5)	0.8 (0.2)
	- *Cerastoderma edule*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- *Hydrobia ulvae*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- *Liocarcinus holsatus*	0.0 (0.0)	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)	0.1 (0.1)	0.1 (0.0)
	- *Littorina littorina*	0.0 (0.0)	0.1 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.0)
	- *Macoma balthica*	0.0 (0.0)	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)	0.1 (0.0)	0.1 (0.0)
	- *Myoxocephalus scorpius*	0.0 (0.0)	0.1 (0.1)	4.1 (4.1)	0.0 (0.0)	0.0 (0.0)	0.5 (0.5)
	- *Mytilus edulis*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.1 (0.0)	0.0 (0.0)
	- *Nereis virens*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 (0.2)	0.1 (0.0)
	- *Pholis gunnulus*	0.0 (0.0)	1.1 (1.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.5 (0.5)
	- *Sprattus sprattus*	0.1 (0.1)	0.8 (0.4)	0.0 (0.0)	20.1 (0.0)	0.9 (0.3)	0.9 (0.4)
Mixture *	Total	61.5 (8.1)	32.4 (3.7)	9.6 (3.6)	32.3 (19.3)	12.6 (3.6)	28.7 (2.7)
	Gasterosteus aculeatus	61.4 (8.1)	23.3 (3.0)	8.0 (3.3)	0.0 (0.0)	4.2 (1.3)	21.6 (2.5)
	Osmerus eperlanus	0.0 (0.0)	2.9 (1.3)	0.0 (0.0)	0.0 (0.0)	3.3 (1.6)	2.2 (0.7)

Year: 2012–2013		Texel (n = 18)	Vlieland (n = 60)	Terschelling (n = 15)	Ameland (n = 2)	Schiermonnikoog (n = 33)	Totals (n = 128)
Habitat	Prey item	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)
Mixture *	Other ‘mixture’ prey *	0.1 (0.1)	6.1 (2.4)	1.6 (1.2)	32.3 (19.3)	5.1 (2.7)	4.9 (1.4)
	- *Anguilla anguilla*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	4.6 (2.7)	1.2 (0.7)
	- *Atherina presbyter*	0.0 (0.0)	0.2 (0.1)	0.0 (0.0)	0.0 (0.0)	0.4 (0.4)	0.2 (0.1)
	- *Palaemon* sp.	0.1 (0.1)	2.4 (1.6)	1.6 (1.2)	32.3 (19.3)	0.0 (0.0)	1.8 (0.9)
	- *Platychtys flesus*	0.0 (0.0)	1.6 (1.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.7 (0.7)
	- *Zoarces viviparous*	0.0 (0.0)	1.9 (1.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.9 (0.5)
Freshwater	**Total**	**4.2 (4.2)**	**11.8 (3.3)**	**18.7 (7.3)**	**0.0 (0.0)**	**17.5 (4.5)**	**12.8 (2.2)**
	Perca fluviatilis	0.0 (0.0)	1.9 (0.9)	0.9 (0.5)	0.0 (0.0)	6.1 (2.1)	2.6 (0.7)
	Pungitius pungitius	0.0 (0.0)	4.4 (1.9)	0.5 (0.5)	0.0 (0.0)	0.0 (0.0)	2.1 (0.9)
	Rutilus rutilus	4.2 (4.2)	3.8 (1.4)	14.7 (7.2)	0.0 (0.0)	7.9 (3.0)	6.1 (1.5)
	Other freshwater prey	0.1 (0.0)	1.8 (1.1)	2.6 (2.0)	0.0 (0.0)	3.5 (1.6)	2.1 (0.7)
	- *Abramis brama*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- *Acilius sulcatus*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- *Blicca bjoerkna*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- *Callicorixa* sp.	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- Coleoptera	0.0 (0.0)	0.1 (0.0)	0.1 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- Copepoda	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- *Corixa punctate*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- Corixa sp.	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- *Dytiscus marginalis*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- *Esox lucius*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- *Gobio gobio*	0.0 (0.0)	0.5 (0.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.2 (0.2)
	- *Graphoderus* sp.	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- *Gymnocephalus cernuus*	0.0 (0.0)	1.1 (0.9)	2.5 (2.0)	0.0 (0.0)	1.9 (0.7)	1.3 (0.5)
	- *Notonecta glauca*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	- *Orconectes limosus*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.6 (1.1)	0.4 (0.3)
	- *Sander lucioperca*	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

* Prey categorized under ‘mixture’ is prey that occurs in marine, brackish and freshwater habitat types.

difference to the contributions of marine prey to the diet (mean difference 3.01 ±2.63 %). During the whole nestling period, spoonbill nestlings are mainly fed with marine prey, except for chicks on Texel who mainly had been fed sticklebacks on the day of capture (Figure 5.4A) after having been fed a lot of shrimp in the previous weeks

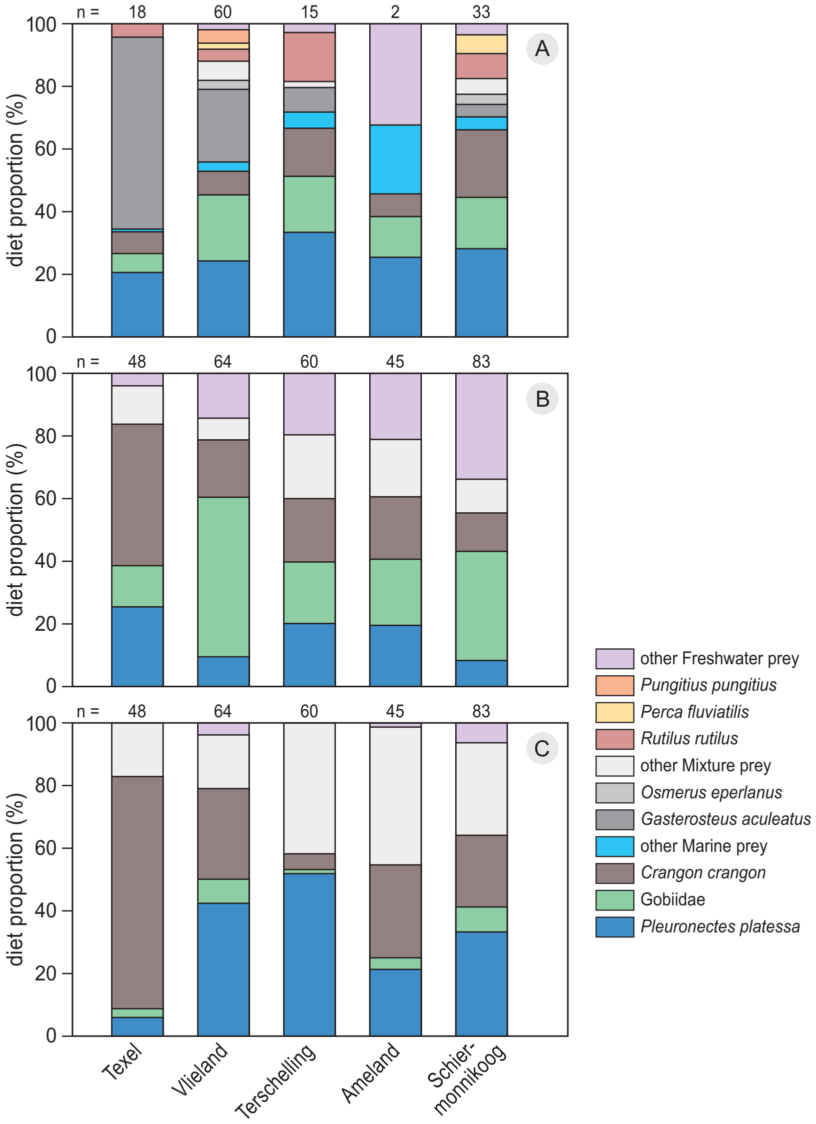


Figure 5.4: Diet of nestling spoonbills on the different Wadden Sea islands based on (A) regurgitate analysis, (B) stable isotope analysis on plasma and (C) stable isotope analysis on RBC.

(Figure 5.4C). Whereas the contribution of flatfish in the diet did not differ between colonies, the contribution of three-spined stickleback decreased from west to east (Figure 5.4A, ANOVA $_{\text{Flatfish}}$; $F_{(4,\,123)} = 0.668$, $P = 0.615$, ANOVA $_{\text{Stickleback}}$; $F_{(4,\,123)} = 23.34$, $P < 0.001$). The contribution of gobies varied significantly between islands (Figure 5.4A, ANOVA $_{\text{Gobiidae}}$; $F_{(4,\,123)} = 2.51$, $P = 0.045$) although without the data for Texel, the contribution of gobies was uniform (ANOVA $_{\text{Gobiidae without Texel}}$; $F_{(3,\,106)} = 0.489$, $P = 0.690$). The isotope-based diet reconstructions confirmed that nestling spoonbills were mainly fed marine prey, with fish (mainly gobies and flatfish) and brown shrimp contributing most to the diet (Figure 5.4B,C).

Discussion

As expected, most prey delivered to growing spoonbill chicks on the barrier islands had a marine origin, indicating that they were caught in the Wadden Sea by the provisioning parents. This means that the growth of chicks is 'fuelled' by local prey resources, rather than resources from afar (e.g. found in freshwater habitats on the mainland). Herring gulls *Larus argentatus* breeding in the same areas have been shown to sometimes provision chicks with freshwater food item collected far away in inland areas (Bukacinska et al., 1996). According to a study by El-Hacen et al. (2014), who reconstructed their diet based on feather isotopes, freshwater prey are the main food source for spoonbill chicks on Schiermonnikoog early in the breeding season, being replaced by marine items later on, matching the time of the year this study was carried out. For chicks born in June-July 2010, El-Hacen et al. (2014) found a contribution of brown shrimp of 37%, more than the SIAR estimates of 23% based on the isotope signature of RBC in the present study (Figure 5.4C, Schiermonnikoog).

The finding that flatfish and gobies were the main marine prey species in the Wadden Sea was an unexpected result. After all, the available diet assessments of spoonbills in the Wadden Sea, based on what was taken as 'common knowledge' (Wintermans et al., 1996; Altenburg et al., 1997; Hollander, 1997), visual observations of ingested food items (van Wetten et al., 1986a,b), visual examination of the stomach content of a single dead spoonbill (Tinbergen, 1933), or direct observations of prey found in feeding areas (Kemper, 1986a,b; van Wetten et al., 1986a,b), all stated that brown shrimp would be the main prey. Indeed, the colour and structure of regurgitates beguilingly suggest brown shrimp to be the main component; this is due to the low digestibility of the shrimps' chitin exoskeletons (Jackson et al., 1992) compared with the fish meat which is more rapidly digested by the spoonbills.

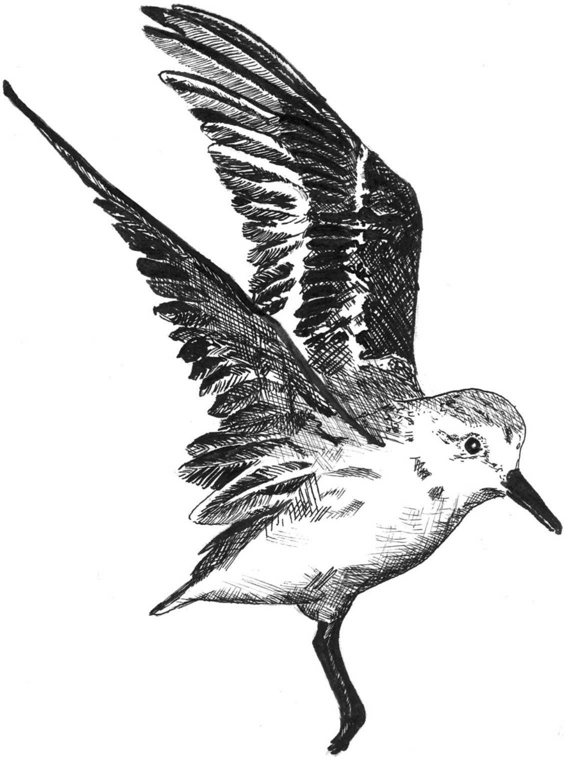
Our analysis rectifies the notion that shrimp are the main marine prey (at least for the chicks), and suggests that small fish rather than brown shrimp contribute most to the spoonbill nestling diets. Our finding is consistent with prey preference experiments with a captive second-year spoonbill reported in the grey literature by van Wetten et al. (1986a). When simultaneously offered fish and shrimp, spoonbill preferred fish (van Wetten et al., 1986a). This may be explained by their higher digestibility (Jackson et al. 1992), higher biomass per prey item, and possibly smaller handling times (van Gils et al., 2005). Also, unlike marine fish, shrimp are isotonic with sea water (Spaargaren, 1971), yielding a salt load that spoonbills may try to avoid (Gutiérrez, 2014; Gutiérrez and Piersma, 2016).

From the late 1980s onwards, the Wadden Sea lost a substantial part of its important function as a nursery for flatfish (van der Veer et al., 2011), with small populations of the young age classes of plaice lingering on. Long-term trends in the western Wadden Sea intertidal area are consistent with this view, with a decrease of juvenile flatfish abundance, but without clear trends for gobies and brown shrimp (Jung et al., 2017). Furthermore the stocks of adult shrimp in the deeper parts across the Wadden Sea first generally increased (Tulp et al., 2012) , followed by a decrease again due to overfishing (Tulp et al., 2016). In view of their preference to provision their chicks with fish rather than shrimp, we suggest that their preferred prey (flatfish) being scarce in recent years will have been the most important factor leading to density-dependent recruitment declines of growing spoonbill colonies and the rapid saturation of colony sizes in the Wadden Sea (Oudman et al., 2017). During the initial phase of their population recovery (1965-1990; Lok et al., 2013), spoonbills might actually have been benefited by the favourable food conditions in the form of an abundance of juvenile flatfish and gobies rather than mature brown shrimp (van der Veer et al., 2011), compared to relatively greater densities of brown shrimps recently.

The current levelling off of the growth of the spoonbill population breeding on the Wadden Sea barrier islands (Oudman et al., 2017) is associated with low stocks of their favourite small fish prey (van der Veer et al., 2011). A preference for small fish rather than shrimp would make colony growth characteristics a good indicator of the abundance of small fish in the Wadden Sea. This is a state of affairs that appears comparable to that of the harbour seals (*Phoca vitulina*) in the Dutch Wadden Sea where the levelling off is also explained by limited access to (larger) fish (Brasseur, Reijnders, Cremer et al. 2018). We predict that successful (fishery) management towards recovery of the former densities of young flatfish, or an increase of small and juvenile fish abundance in general, will be tracked by changing spoonbill diets (more fish), improved breeding success, and an increasing size of the spoonbill colonies across the Wadden Sea.

Acknowledgements

This study would not have been possible without the participation of Eric Menkveld, Carl Zuhorn, Arjan Zonderland, Oene de Jong, Richard Kiewiet, the late Frits Oud, and Erik Jansen (nature managers on the Wadden islands of It Fryske Gea, Natuurmonumenten and Staatsbosbeheer) and all spoonbill volunteers, especially Harry Horn and Roos Kentie. We thank Rebecca Reurslag for her help with the regurgitate analyse; Stefan Schouten, Thomas Leerink and Kevin Donkers (NIOZ) for technical assistance in stable isotope analyses and Sarina Jung for the population trends of Gobiidae. We are grateful to Stefan Schouten for giving us advanced isotopic advice. Obeying the Dutch laws, field work was carried out under welfare (DEC) protocol RuG-4752 amendement D. This study was carried out as part of the projects 'Waddensleutels' (WF203930) and 'Metawad' (WF209925), both supported by Waddenfonds.



CHAPTER 6

Ecological forensics: Using single point stable isotope values to infer seasonal schedules of animals after two diet switches

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Abstract

1. Animals adjust to seasonal challenges in physical, behavioural and spatial ways. Such adjustments are commonly associated with diet changes that often can be characterized isotopically.
2. We introduce the 'double diet switch model', with which the occurrence and timing of two subsequent diet switches of an individual animal can be traced with a single sample assayed for stable isotopes. We demonstrate the model for Sanderling, Calidris alba, a small shorebird that migrates from the Nearctic tundra breeding grounds to the intertidal flats of the Wadden Sea; during this migration some birds may stage in the North Atlantic areas.
3. The 'double diet switch model' successfully predicted the occurrence and timing of two diet switches in 59 Sanderlings captured in the Wadden Sea in July-September. Excluding birds that likely had over-summered at North Atlantic staging areas, the model predicted that Sanderlings departed from the Arctic on 13 July (range: 9–17 July), had a staging duration of 18.6 days in the North Atlantic, and arrived in the Wadden Sea on 1 August (31 July – 1 August).The estimated mean Arctic departure dates coincided with the mean hatching date, suggesting that many individuals failed to produce young or left the care to a partner. Estimated mean arrival date matched the main arrival period in the Wadden Sea obtained from observation data. In this study we did not use lipid-free tissues, which may bias model predictions. After correcting for lipid components, the estimated departure date was 11 days later and the staging duration 8.5 days shorter, while arrival date was similar.
4. The 'double diet switch model' successfully identified the occurrence and timing of two subsequent diet switches. The 'double diet switch model will not only apply to switches between three isotopic levels (as in the case study on Sanderling) but also to scenarios where the second switch reverses to the initial isotopic level. Due to this general applicability, the model can be adapted to a wide range of taxa and situations. Foreseeable applications include changes in habitat and food type, ontogenetic development, or drastic phenotypic changes such as the metamorphosis in insects and amphibians.

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Introduction

Animals adjust to seasonal challenges by movements and by physical and behavioural changes (Piersma & van Gils 2011). Quite commonly, these adjustments are associated with diet changes that can be isotopically characterized (Hobson 1999; Caut, Angulo & Courchamp 2009). The accompanying shifts in isotopic value enables researchers to illuminate seasonal phenomena such as migration, metamorphosis, or (temporary) increasing or declining food availability (Phillips & Eldridge 2006; Karasov & Martínez del Rio 2007; Schwemmer et al. 2016). No surprise that 'ecological forensics' is thriving (Dawson & Siegwolf 2011).

Stable isotope analyses can track the occurrence and timing of diet switches based on differences in (1) isotopic values generated by foraging on isotopically distinct food sources and (2) incorporation times of an isotope in distinct consumer tissues (e.g. plasma and red blood cells: Hobson 1999; Klaassen et al. 2010). After a diet switch, the isotopic incorporation of the new diet in a consumer's tissues follows a first order kinetics model, mostly described by an exponential decay function. This model can estimate the time since a single diet switch by using stable isotope values of one, or preferably two, tissue types (Phillips & Eldridge 2006; Klaassen et al. 2010; Oppel & Powell 2010). For animals that change their foraging location or diet more than once over relatively short time spans, we here describe a 'double diet switch model'. This model can deal with three successive isotopically distinct diets based on a single assessment of isotopic values in two tissues with distinct turnover rates in one individual and gives estimates of the timing of the two consecutive diet switches.

To demonstrate the functionality of the model, we estimate the timing of post-breeding migration of Sanderlings *Calidris alba* upon their arrival in the Dutch Wadden Sea. After a breeding season in the High Arctic, these long-distance migratory shorebirds depart from the tundra where they fed on terrestrial arthropods (Wirta et al. 2015). Before arrival in the Wadden Sea, where they mainly feed on Brown Shrimp *Crangon crangon* (JR pers. comm.), Sanderlings may or may not make refuelling stops in coastal habitats in the North Atlantic where soft-bodied marine invertebrates comprise the diet (Reneerkens et al. 2009).

Methods

The double diet switch model

The isotopic change of body tissues after a diet switch typically follows a first-order kinetic response which is generally well described by a negative exponential function (Tieszen et al. 1983; Phillips & Eldridge 2006; Klaassen et al. 2010). Specifically, consider a focal animal on a diet A, with a corresponding isotope ratio δ_{A1} in tissue 1. If at time $t = 0$ the animal switches from diet A to diet B, then after t_B days on the new diet, its tissue-specific isotope ratio is given by the formula

$$\delta(t_B) = \delta_{B1} + (\delta_{A1} - \delta_{B1})e^{-\lambda_1 t_B}, \tag{1}$$

where δ_{B1} is the characteristic isotope ratio of diet B in issue 1, and λ_1 is the tissue-specific turnover rate (1/day) of the isotope. Given estimates of δ_{A1}, δ_{B1} and λ_1, this 'single diet switch model' allows estimation of t_B, the amount of time since the diet switch occurred (Phillips & Eldridge 2006; Klaassen et al. 2010).

Here we expand this 'single diet switch model' to one which describes two diet switches: the 'double diet switch model'. Suppose that at time $t = t_B$, our focal animal switches once again, from diet B to diet C, the latter having characteristic isotopic ratio δ_{C1} in tissue 1. After t_C days on diet C, at time $t = t_B + t_C$, the animal's isotope ratio is now given by

$$\begin{aligned} \delta(t) &= \delta_{C1} + [\delta(t_B) - \delta_{C1}]e^{-\lambda_1 t_C} \\ &= \delta_{C1} + [\delta_{B1} + (\delta_{A1} - \delta_{B1})e^{-\lambda_1 t_B} - \delta_{C1}]e^{-\lambda_1 t_C}, \end{aligned} \tag{2}$$

where we substituted the right-hand side of formula (1) for $\delta(t_B)$ in the first line. Note that this formula is not very useful by itself, since any observed value of $\delta(t)$ within the range spanned by δ_{A1}, δ_{B1} and δ_{C1} is typically consistent with infinitely many combinations of t_B and t_C. However, if a sample is taken simultaneously from a second tissue with a different turnover rate λ_2, then we have a system of two equations for the two unknowns t_B and t_C:

$$\begin{aligned} \delta_1(t) &= \delta_{C1} + [\delta_{B1} - \delta_{C1} + (\delta_{A1} - \delta_{B1})e^{-\lambda_1 t_B}]e^{-\lambda_1 t_C} \\ \delta_2(t) &= \delta_{C2} + [\delta_{B2} - \delta_{C2} + (\delta_{A2} - \delta_{B2})e^{-\lambda_2 t_B}]e^{-\lambda_2 t_C} \end{aligned} \tag{3}$$

Geometrically, the two equations correspond to two curves in the t_B–t_C plane, and solutions to the two equations occur if and where the curves intersect. As we shall see below, these solutions are precisely the maximum likelihood estimates of t_B and t_C, provided that $\delta_1(t)$ and $\delta_2(t)$ are normally distributed around their predicted values. Solving both equations for t_C gives explicit formulas for the two curves:

$$\begin{aligned} t_C &= \frac{1}{\lambda_1} \ln \frac{\delta_{C1} - \delta_{B1} + (\delta_{B1} - \delta_{A1})e^{-\lambda_1 t_B}}{\delta_{C1} - \delta_1(t)} \\ t_C &= \frac{1}{\lambda_2} \ln \frac{\delta_{C2} - \delta_{B2} + (\delta_{B2} - \delta_{A2})e^{-\lambda_2 t_B}}{\delta_{C2} - \delta_2(t)} \end{aligned} \tag{4}$$

Equating both right-hand-sides yields an equation in t_B, which does not have closed-form solutions, but which may be solved by standard numerical routines. If a solution is found, it can be put back into either of the right-hand sides of (4) to give a corresponding solution for t_C.

Thus, the 'double diets switch model' allows estimation of seasonal scheduling of animals with three subsequent diets, such as migrant birds consuming isotopically distinct diets before the start of migration, during a staging episode and after arrival to final destination, respectively, or grizzly bears (*Ursus arctos*) switching temporarily from a diet with mainly whitebark pine (*Pinus albicaulis*) to a diet with mainly elk (*Cervus elaphus*) (Schwartz et al. 2014). The conditions for the use of the 'double diet switch model' are presented in table 6.1. In the next section we describe a statistical method to estimate t_B and t_C.

Table 6.1: An overview of the required conditions for the 'double diet switch model' to estimate the timing of two consecutive diet switches

Conditions for using the 'double diet switch model':

a) Stable isotope analysis (e.g. $\delta^{13}C$) of individuals of the study species should be measured while on the third diet. This should be done for two tissue types: one with a relatively high turnover rate such as plasma and one with a relatively low turnover rate such as RBC). Both tissues should be sampled at the same moment. Tissue sampling needs to be performed before the individual has reached isotopic adaptation of the new equilibrium of the third diet.

b) At all three stages (or locations), the stable isotope values of the study species itself or that of its food are known (plus a discrimination factor; but see supporting information I) and sufficiently distinct from each other.
Ideally, stable isotope values are known for both separate tissue types at all three stages.

c) Turnover rates of the two tissues are known for the study species (or can be estimated sufficiently accurately).

d) Preferably, sampling dates of the tissue types are known. With this information durations of the use of a diet can be transferred to dates instead of number of days.

e) Diet uniformity among individuals.

f) The sampling moment is important, since there should not have been enough time to approach equilibration to diet C. Besides, the animal's staging duration should be shorter than the time to approach the equilibration to diet B.

The likelihood model

We use a maximum likelihood (ML) approach to estimate the parameters t_B and t_C in the nonlinear model (3), given estimates of all other parameters and the measured values of δ_1 and δ_2. We assume that measurement errors have a normal density:

$$p(\delta_1,\delta_2 \mid t_B,t_C) = \frac{1}{2\mu\sigma_\delta^2} \exp -\frac{1}{2\sigma_\delta^2}((\delta_1 - \mu_1)^2 + (\delta_2 - \mu_2)^2) \ . \tag{5}$$

Here σ_δ^2 is the variance, assumed known and identical for both tissues, while μ_1 and μ_2 are the expected values of δ_1 and δ_2 according to model (3):

$$\begin{aligned} \mu_1(t_B,t_C) &= \delta_{C1} + [\delta_{B1} - \delta_{C1} + (\delta_{A1} - \delta_{B1})e^{-\lambda_1 t_B}]e^{-\lambda_1 t_C} \\ \mu_2(t_B,t_C) &= \delta_{C2} + [\delta_{B2} - \delta_{C2} + (\delta_{A2} - \delta_{B2})e^{-\lambda_2 t_B}]e^{-\lambda_2 t_C} \end{aligned} \tag{6}$$

The log-likelihood is then, up to a constant term:

$$1(t_B,t_C) = -\frac{1}{2\sigma_\delta^2}((\delta_1 - \mu_1)^2 + (\delta_2 - \mu_2)^2) \tag{7}$$

The score, the partial derivatives of the log-likelihood with respect to both parameters is then given by

$$\begin{aligned} \frac{\partial 1}{\partial t_B} &= \frac{1}{\sigma_\delta^2}(\delta_1 - \mu_1)\frac{\partial \mu_1}{\partial t_B} + (\delta_2 - \mu_2)\frac{\partial \mu_2}{\partial t_B} \\ \frac{\partial 1}{\partial t_C} &= \frac{1}{\sigma_\delta^2}(\delta_1 - \mu_1)\frac{\partial \mu_1}{\partial t_C} + (\delta_2 - \mu_2)\frac{\partial \mu_2}{\partial t_C} \end{aligned} \tag{8}$$

Clearly the score vanishes if $\mu_1 = \delta_1$ and $\mu_2 = \delta_2$, which shows that the ML estimates of t_B and t_C are indeed the solutions to the system of equations (4). We used the function uniroot in R version 3.3.0 (R Core Team 2016) to find numerical solutions. All R scripts are available as online appendices to this paper.

The Hessian matrix of second order derivatives, evaluated at the candidate ML estimates, is

$$H = \begin{matrix} \frac{\partial^2 1}{\partial t_B^2} & \frac{\partial^2 1}{\partial t_B \partial t_C} \\ \frac{\partial^2 1}{\partial t_B \partial t_C} & \frac{\partial^2 1}{\partial t_C^2} \end{matrix} = \frac{1}{\sigma_\delta^2} \begin{matrix} -{\frac{\partial \mu_1}{\partial t_B}}^2 - {\frac{\partial \mu_2}{\partial t_B}}^2 & -\frac{\partial \mu_1}{\partial t_B}\frac{\partial \mu_1}{\partial t_C} - \frac{\partial \mu_2}{\partial t_B}\frac{\partial \mu_2}{\partial t_C} \\ -\frac{\partial \mu_1}{\partial t_B}\frac{\partial \mu_1}{\partial t_C} - \frac{\partial \mu_2}{\partial t_B}\frac{\partial \mu_2}{\partial t_C} & -{\frac{\partial \mu_1}{\partial t_C}}^2 - {\frac{\partial \mu_2}{\partial t_C}}^2 \end{matrix} \tag{9}$$

The Hessian has two uses here: first, to verify that candidate ML solutions are indeed maxima of the likelihood, and secondly, to provide approximate standard errors for the ML estimates. A local maximum is verified if $\mathrm{tr}(H) = H_{11} + H_{22} < 0$, which is easily seen to be true, and if $\det(H) = H_{11}\,H_{22} - H_{12}\,H_{21} > 0$, which is also true since

$$\det(H) = \left(\frac{\partial\mu_1}{\partial t_{\mathrm{B}}}\frac{\partial\mu_2}{\partial t_{\mathrm{C}}} - \frac{\partial\mu_1}{\partial t_{\mathrm{C}}}\frac{\partial\mu_2}{\partial t_{\mathrm{B}}}\right)^2 > 0.$$

Approximate standard errors and covariances for the ML estimates $\hat{t}_{\mathrm{B}}$ and $\hat{t}_{\mathrm{C}}$ follow from

$$-H^{-1} \approx \begin{matrix} \sigma^2_{\hat{t}_{\mathrm{B}}} & \sigma_{\hat{t}_{\mathrm{B}}\hat{t}_{\mathrm{C}}} \\ \sigma_{\hat{t}_{\mathrm{B}}\hat{t}_{\mathrm{C}}} & \sigma^2_{\hat{t}_{\mathrm{C}}} \end{matrix} \tag{10}$$

The matrix $-H$ is called the information matrix, since the inverse of information is uncertainty, as quantified by standard errors. To evaluate H we need to evaluate the partial derivatives for tissues $i = 1, 2$:

$$\begin{aligned} \left.\frac{\partial\mu_1}{\partial t_{\mathrm{B}}}\right|_{t_{\mathrm{B}}=\hat{t}_{\mathrm{B}},t_{\mathrm{C}}=\hat{t}_{\mathrm{C}}} &= -\lambda_i\,(\delta_{\mathrm{A}i} - \delta_{\mathrm{B}i})e^{-\lambda_i(\hat{t}_{\mathrm{B}}+\hat{t}_{\mathrm{C}})} \\ \left.\frac{\partial\mu_1}{\partial t_{\mathrm{C}}}\right|_{t_{\mathrm{B}}=\hat{t}_{\mathrm{B}},t_{\mathrm{C}}=\hat{t}_{\mathrm{C}}} &= -\lambda_i\,((\delta_{\mathrm{B}i} - \delta_{\mathrm{C}i}) + (\delta_{\mathrm{A}i} - \delta_{\mathrm{B}i})e^{-\lambda_i\hat{t}_{\mathrm{B}}})e^{-\lambda_i\hat{t}_{\mathrm{C}}} \end{aligned} \tag{11}$$

Plugging these into (9) clearly shows that the uncertainty about $\hat{t}_{\mathrm{B}}$ and $\hat{t}_{\mathrm{C}}$ increases exponentially with their estimated mean values. Specifically, according to the first equation in (11), information regarding t_{B} decays exponentially if either t_{B} or t_{C} grows large, while according to the second equation information regarding t_{C} is especially sensitive to large t_{C} but not t_{B} values. Thus, unless turnover rates are very low, it is clearly preferable to sample not too long after the second diet switch, nor should the time between diet switches be too long.

We have attempted to take a full Bayesian approach to estimate t_{B} and t_{C}, but the maximum likelihood (ML) approach was superior. Simulations indicated (results not shown) that even weakly informative priors produced considerable bias in estimates. The use of flat priors is ruled out for our model since the likelihood does not converge to zero as t_{B} and t_{C} go to infinity, rendering the corresponding posterior distribution non integrable.

Sensitivity analysis

The model has 8 parameters: for each tissue $i = 1, 2$ and diet $j = \mathrm{A, B, C}$ the equilibrium isotope ratios are denoted by δ_{ij} and turnover rates by λ_i. For the Sanderling data, the diet-and tissue-specific isotope ratios and associated standard deviations were estimated directly from blood and indirectly from prey items (table 2, supporting information I). No direct information about turnover rates was available for the Sanderling. Instead values for λ_i were predicted on the basis of interspecific allometric regressions, while standard deviations were obtained as averages of intraspecific standard deviations (table S1, supporting information II).

To assess the sensitivity of model predictions to uncertainty in the 8 parameters, for each bird in our dataset we drew 10000 random normal deviates for each of the 6 isotope ratios and for the logarithms of the turnover rates (which must be positive), based on our estimates of mean values and standard deviations. For the isotope ratios we used independent draws, while for turnover rates we allowed for a positive correlation between tissues since it seems plausible that variation in metabolic rate affects turnover rates in the same direction. For each of the draws we attempted to obtain ML estimates for t_B and t_C by solving system (4). When we obtained a candidate solution, we calculated the Hessian to verify it corresponded to a maximum and to estimate standard errors for the parameter estimates. Thus, for each bird we obtained 1000 distributions, one for each successful random draw, which we approximated as a mixture of 10000 gamma distributions to avoid negative values in the tails of the distributions. The mixture was stored as a "posterior distribution" from which we calculated mean values and 89% highest posterior density intervals.

As an alternative to our simulation approach to sensitivity analysis, parameter likelihoods may also be incorporated into an overall likelihood for all model parameters, in addition to t_B and t_C, and corresponding confidence levels calculated. Such an extended likelihood-approach would have to be tailored to the study-specific way the additional parameters were estimated.

The case: timing of southward migration in sanderling

Using the 'double diet switch model', we reconstructed the timing of southward migration by Sanderlings from the tundra breeding grounds (where they ate diet A) and subsequently flew, with or without staging in the North Atlantic (diet B), to the Wadden Sea (diet C). In July-September 2011 and 2012, 65 adult Sanderlings were captured with mist-nets during new moon nights near high-tide roosts in the western Dutch Wadden Sea (53°N, 4-5°E). In addition, 10 adult Sanderlings were caught on their nests in Greenland (Zackenberg, 74°30'N, 21°00'W) in the second half of June 2009. Blood samples of these latter birds were used to determine the $\delta^{13}C$ value of red blood cells (RBC) and plasma of birds on the initial diet in the Arctic (diet A; see supporting information I). Immediately after capture, all 75 Sanderlings were (colour)-ringed, weighed and aged based on plumage criteria (Prater, Marchant & Vuorinen 1977), and a blood sample (~300 μL) for stable isotope analysis was drawn from the brachial vein into heparinised capillaries. Note that second calendar year Sanderlings cannot be distinguished from older Sanderling based on their plumage after their first basic moult in spring (Prater, Marchant & Vuorinen 1977; Lemke, Bowler & Reneerkens 2012). Immediately after sampling, the blood was centrifuged in Eppendorf cups in a haematocrit centrifuge (microfuge Sigma 1–13, 6 min on 5000 rpm). Plasma and RBC were pipetted in separate glass vials and stored in a freezer (–20°C) until analysis.

The Sanderling dataset serves all conditions for the 'double diet switch model', as described in Table 6.1: (a) Stable carbon isotope analysis were performed on plasma and RBC of Sanderlings caught in the Wadden Sea. (b) The $\delta^{13}C$ values of plasma and RBC of Sanderlings differed between all three locations along the migration route

Table A2: Summary of all general input variables of the 'double switch model' to estimates individual schedules in migrating Sanderling. Presented are the $\delta^{13}C$ values of Sanderling in equilibrium with the diets on the three locations along southward migration (mean ±SE). The $\delta^{13}C$ values were calculated in two ways and shown in two columns: obtained from Sanderling blood (True) and a calculated value with help of $\delta^{13}C$ values of prey and a discrimination factor (Calc.). The results of the two methods did not differ significantly (see t-test in last column and supporting information I). Bold values were used in the model.

Diet	Tissue type	Calc. (prey +DiF)[4]	n	True (bird blood)	n	t-test
Arctic[1]	plasma			−25.99±0.29 ‰	10	
	RBC			−25.33±0.29 ‰		
Staging area[6]	plasma	−18.29 ±0.24 ‰	25	−18.28 ±0.14[2] ‰	4	t(27) = 0.02, P = 0.99
	RBC	−17.62 ±0.24 ‰		−17.94 ±0.30[2] ‰		t(27) = 0.53, P = 0.60
Wadden Sea	plasma	−14.56 ±0.09 ‰	20	−14.54 ±0.16[3] ‰	6	t(24) = 0.16, P = 0.91
	RBC	−13.90 ±0.09 ‰		−13.94 ±0.13[3] ‰		t(24) = 0.23, P = 0.82

Turnover rate[5]	Tissue type	Mean	SD
	plasma	0.303	0.033
	RBC	0.056	0.012

[1] Based on blood of Sanderlings caught in northeast Greenland.

[2] Blood of Sanderlings caught in Wadden Sea in summer with $\delta^{13}C$ values of plasma and RBC that both represented the staging location ($\delta^{13}C_{plasma}$ minus $\delta^{13}C_{RBC}$ <0.23 ‰). These birds were suspected to have just arrived in the Wadden Sea after using a staging area somewhere in the North Atlantic.

[3] Blood of Sanderlings caught in September in the Wadden Sea with $\delta^{13}C$ values of plasma and RBC that both represented the Wadden Sea ($\delta^{13}C_{plasma}$ minus $\delta^{13}C_{RBC}$ <0.23 ‰).

[4] See supporting information I for details about indirect calculations of the $\delta^{13}C$ signal of Sanderlings. DiF = discrimination factor.

[5] See supporting information II for calculation of the turnover rate of $\delta^{13}C$ in plasma and RBC of Sanderlings.

[6] North Atlantic staging area

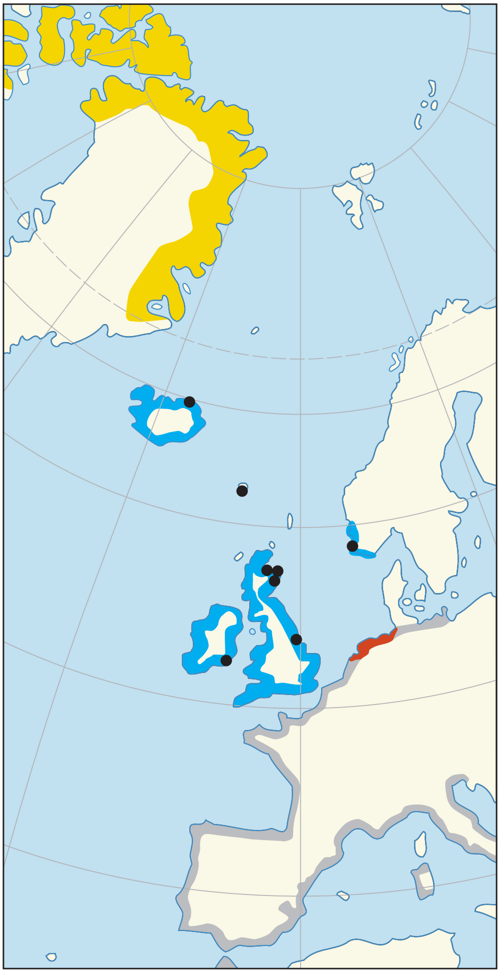


Figure 6.1: Arctic breeding areas (yellow), North Atlantic staging areas (blue) and the Wadden Sea (red) used by Sanderlings visiting the Wadden Sea in late summer. Known wintering areas are shown in grey, but the Wadden Sea area (red) is a wintering area too. The coastal North Atlantic staging areas were determined based on observations of eight colour-ringed Sanderlings (black dots in blue area) that were observed in the Dutch Wadden Sea a few days later.

(Table 6.2). North Atlantic staging areas were assigned based on eight re-sightings of colour-ringed Sanderlings (2007–2014) recorded within the same season of southward migration at both a North Atlantic staging area and the Wadden Sea (Figure 6.1). The isotope values of Sanderling's RBC and plasma at locations A and C were obtained from Sanderling blood samples, while the isotope values of RBC and plasma at the North Atlantic staging location (location B) were estimated via prey tissues and a discrimination factor (see supporting information I). (c) The turnover rates for plasma ($\lambda_{plasma} = 0.303 \pm 0.033$ SD) and RBC ($\lambda_{RBC} = 0.056 \pm 0.012$ SD) were estimated for an average adult Sanderling (see supporting information II). (d) The tissue sampling dates of all Sanderlings captured in the Wadden Sea were known. (e) There is no indication for non-uniformity in diet between individual Sanderlings under any of the three diets. Besides, it is unlikely that individual diet specialisation alters the average stable isotope signature of the diet, because we took all important prey species into account, intra-diet variation was within the limits of inter-diet variation, and the consumed prey species differed between the three sites. (f) Samples were collected in the period shortly after the mean arrival period in the Wadden Sea. The ten samples that were collected in late summer, some weeks after the arrival period, indeed showed that the majority of these birds were already adapted to the Wadden Sea diet (Figure 6.2).

Figure 6.2 shows the predictions of the 'double diet switch model' for Sanderlings with different staging durations. The steepness of the slopes of the model predictions increases with turnover rate of the tissue, showing that plasma $\delta^{13}C$ values (dashed lines) adapt more quickly to the new diet than RBC $\delta^{13}C$ values (solid lines). The model is based on the combined differences of values for $\delta^{13}C_{plasma}$, $\delta^{13}C_{RBC}$ and the difference between plasma and RBC isotope values ($\delta^{13}C_{plasma}$ minus $\delta^{13}C_{RBC}$) over time (t_B and t_C). Therefore, the seasonal schedule of an individual Sanderling can be predicted using a single time point measurement of the stable isotopic value of two tissues. Birds with an Arctic isotopic value in both RBC and plasma are still in equilibrium with the Arctic diet and must have flown directly to the Wadden Sea. Birds with a very short staging period in the North Atlantic staging area and recently arrived in the Wadden Sea will also show a predominantly Arctic signature. Birds with Wadden Sea isotopic values in both RBC and plasma are birds that have been long enough in the Wadden Sea for both tissues to achieve equilibrium with the Wadden Sea diet. We expect that the 'double diet switch model' cannot assign a staging duration to Sanderlings that are already isotopically resident in the Wadden Sea (cf. Hobson 1999). Birds with intermediate values might have been in the Wadden Sea for some time, but not long enough to be in equilibrium with the Wadden Sea diet, and/or may have staged in the North Atlantic region.

Note that migratory flights from the Arctic breeding area in Greenland to the Wadden Sea, which we expect to last approximately two days (65 km/h ground speed for the whole flight of approx. 2850 km; Zwarts et al. 1990), are not taken into account in the model. Although this could potentially affect the biological interpretation of departure dates from the Arctic, the time in flight is short in comparison with the mean error term of t_B (9.1 days, n = 52) . We assumed (1) that a diet switch started upon arrival at a new location and (2) uniform isotopic diets in the three reference

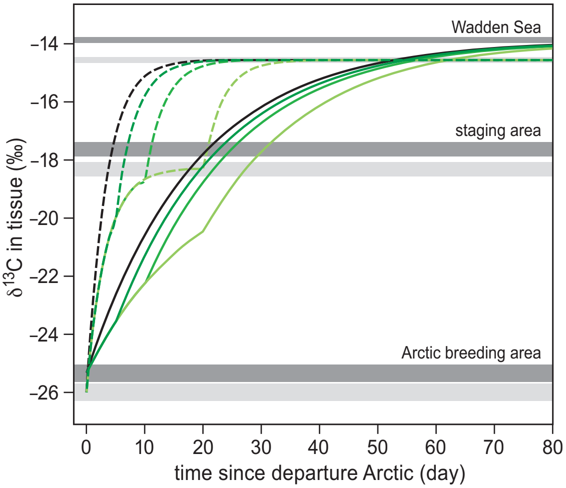


Figure 6.2: Predicted changes in δ^{13}C values in plasma and RBC of Sanderlings with different staging durations during southward migration. The horizontal bars for plasma (light grey) and RBC (dark grey) represent δ^{13}C values in equilibrium with diets used in the Arctic breeding area, the North Atlantic staging area and in the Wadden Sea. The isotopic changes of $\delta^{13}C_{plasma}$ (dashed lines, turnover rate of 0.303) and $\delta^{13}C_{RBC}$ (solid lines, turnover rate of 0.056) are given for staging durations of 0, 5, 10 and 20 days. Black lines show a migration without a stopover. Green lines show migrations with a stopover in the North Atlantic staging area, with colour-darkness corresponding with ascending staging durations.

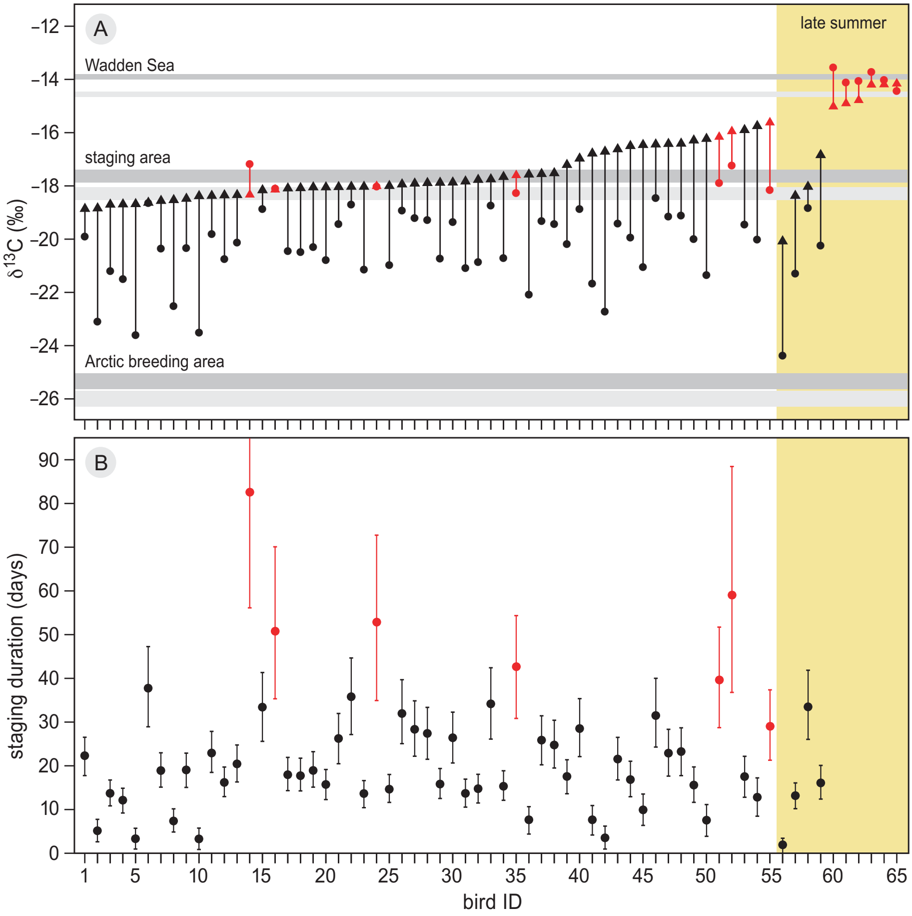
areas are representative for the different regions (Arctic, North Atlantic staging areas and Wadden Sea) used by Sanderlings during southward migration to the Wadden Sea. Note also that output dates were reconstructed from termination of 'day of the year' of 2011, since most birds were caught in that year, while the day of the year differs one day between 2011 and 2012.

To evaluate the seasonal schedules of Sanderlings estimated by our 'double diet switch model', we compared our model data with observation data of seasonal schedules of Greenlandic breeding Sanderlings migrating southwards. In 2007–2014, Sanderling nests were annually searched for in northeast Greenland (Reneerkens et al. 2014). Dates of hatch were often exactly known or, in case of clutch predation, estimated based on egg flotation (Hansen et al. 2011). For families found post-hatch, a body mass growth curve based on local data was used to estimate the hatching date. In total we determined hatching dates of 417 clutches and broods (annual range 25–77). The timing of southward migration of Sanderlings was determined based on sightings of individually colour-ringed birds. More than 5600 Sanderlings were individually marked in 12 countries produced over 58,000 unique observations along the

East Atlantic flyway collected by us and many volunteers. This dataset was used to extract information of birds sighted in the North Atlantic region and the Wadden Sea within the same season of southward migration.

Stable isotope analysis

All bird plasma, RBC and prey items were stored at -20°C before analysis. The samples were freeze-dried before grinding them with a mortar and pestle. We used a microbalance (Sartorius CP2P) to weigh 0.4 – 0.8 mg of the sample material in 5×8 mm tin capsules. The $\delta^{13}C$ values were determined with a Thermo Flash 2000 elemental analyser coupled to a Thermo Delta V isotope ratio mass spectrometer.



Isotope values were calibrated to a laboratory acetanilide standard (δ^{13}C −26.1‰ calibrated on NBS-22) and corrected for blank contribution. 72% of the plasma and RBC samples were analysed in duplicate. The results are reported on the per mill scale with respect to Vienna Pee Dee Belemnite [VPDB]. The replicate error on the standard, acetalinide, ranged between 0.03 and 0.08, using one standard every 4.3 to 7 bird samples.

Elimination of birds oversummering in the North Atlantic region

Out dataset on stable isotope profiles appeared to contain Sanderlings that probably over-summered in the North Atlantic 'staging area' and did not migrate to the Arctic tundra. The estimated staging duration of these individuals was so exceptionally long that if they would have arrived from the Arctic they would have had to depart unrealistically early (as early as 14 May, when Sanderlings are still on northward migration to the Arctic). The 'double diet switch model' cannot eliminate birds that over-summered in the North Atlantic, but simply predicts that these birds have exceptionally long staging durations. In order to eliminate the birds that may have over-summered in the North Atlantic, we excluded birds with a $\delta^{13}C_{RBC}$ that fell within or was higher than the δ^{13}C of the North Atlantic staging area and also had a $\delta^{13}C_{plasma}$ that was still not yet adapted to the Wadden Sea diet (7 birds; see Figure 6.3A).

Figure 6.3 (left): δ^{13}C values of Sanderlings caught in the Wadden Sea after southward migration and their corresponding estimated staging duration along North Atlantic coasts. For clarity, individuals are sorted along the X-axis according to raw δ^{13}C values. Depicting individuals in chronological order of arrival caused many overlaying points because multiple birds were mist-netted per day. Birds in the yellow bar were caught in late summer and represented separately to show the high number of birds that are adapted to the Wadden Sea diet in late summer. (A) Measured values of $\delta^{13}C_{plasma}$ (triangles) and $\delta^{13}C_{RBC}$ (dots) of all 65 individual Sanderlings. Although the model was able to fit a t_B and t_C for all birds, only birds that had been in the Arctic breeding area, indicated with black symbols, were taken into account for further interpretations (n = 52). Birds with a $\delta^{13}C_{RBC}$ within or above the δ^{13}C of the diet of the North Atlantic staging area and a $\delta^{13}C_{plasma}$ that was not already adapted to the Wadden Sea (red symbols) were considered to have over-summered and not used for further interpretations of the migration schedule of Sanderlings. For individuals that were already resident to the Wadden Sea (Late summer, n = 6, table 6.1), the model could (and should) not fit t_B and t_C. (B) The staging duration of all individuals not yet adjusted to the Wadden Sea diet (n = 59) as calculated by the 'double diet switch model'. The confidence limits of the staging duration (t_B) for each individual bird are expressed with standard deviation bars. Again, red symbols indicate birds that likely over-summered in the North Atlantic staging area and therefore were left out for further interpretations of the migration schedule (n = 7). For visualisation we distinguished between birds caught in the main arrival period in summer (23 July – 2 August) and birds caught after the main arrival period in late summer (20 August – 1 September; yellow bar) in the graphs.

Results

The δ^{13}C values of RBC and plasma of Sanderlings caught in the Wadden Sea varied from −24.32 ‰, which is close to a signature of bird's blood in equilibrium with a diet on the Arctic terrestrial arthropods, to −13.5 ‰, which is a signature for bird's blood in equilibrium with the Wadden Sea diet (Figure 6.3A). Whereas most birds captured in late summer showed Wadden Sea diet type isotopic values in both RBC and plasma, birds captured in the main arrival period (23 July to 2 August) showed a variety of patterns ranging from almost purely Arctic signatures, North Atlantic isotopic signatures, intermediate isotopic values, to Wadden Sea diet signatures (Figure 6.3A).

Based on the 'double diet switch model' we assessed the individual seasonal schedules of the Sanderlings (Figure 6.3). Sanderlings had a wide range of migration strategies with staging periods along North Atlantic coasts ranging from 2.2 to 37.6 days (Figure 6.3B). Sanderlings departed from the Arctic on average on 13 July (range: 9–17 July, n = 52, Figure 6.4), to arrive in the Wadden Sea on 1 August (31 July – 1 August, n = 52, Figure 6.4). When we include the seven birds that over-summered in the North Atlantic staging areas, the mean arrival date remained 1 August (range: 31 July – 1 August, n = 59, Figure 6.4). Departure dates from the Arctic and arrival dates in the Wadden Sea for all individual birds are presented in Figure 6.4B.

Discussion

Here we developed a new inferential statistical tool to estimate the timing of movements between distinct habitats on the basis of chemical markers in animal tissues. Ecological forensic problems by their nature are particular and specific, and for this reason we will discuss the Sanderling case before zooming out to the wider range of situations to which our new tool can be applied.

Interestingly, with the help of the 'double diet switch model', we are the first to describe the timing of southward migration of Sanderlings. Our results shows that Sanderlings that spend the summer in the Arctic , as well as those which over-summered in the North Atlantic, arrive simultaneously in the Wadden Sea, matching the main arrival date obtained by observations (Loonstra, Piersma & Reneerkens 2016). As surmised by Reneerkens et al. (2009), the 'double diet switch model' revealed that Sanderlings show large temporal variation in the autumn migration schedules. Contrary to the work of Dietz et al. (2010) who, with the help of a 'single diet switch model' found that Red Knots *Calidris canutus* do not stage in the North Atlantic during southward migration, we show that Sanderlings stage for variable lengths of time in the North Atlantic before moving on the Wadden Sea. The mean staging duration in coastal areas between Greenland and the Netherlands of southward migrating

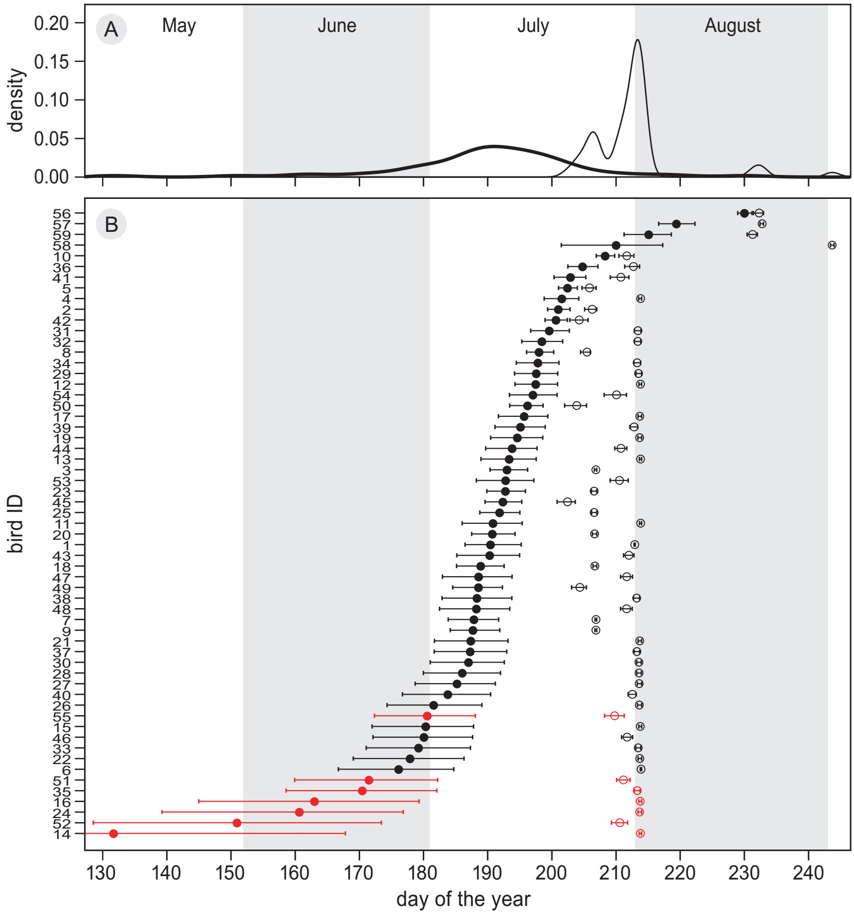


Figure 6.4: Migration schedule of Sanderlings, shown as departure dates from the Arctic and arrival dates in the Wadden Sea. (A) The distribution of the departure date from the Arctic (thick line) and arrival date in the Wadden Sea (thin line), for birds that likely arrived from the Arctic breeding area and thus completed the entire migration (n = 52). The mean departure date from the Arctic is 13 July, the mean arrival date in the Wadden Sea is 1 August. (B) Individual migrating schedules of all 59 Sanderlings with the estimated departure date from the Arctic (filled dots) and the arrival date in the Wadden Sea (open circles), both given as mean ± SD. Black symbols represent birds that likely arrived from the Arctic (n = 52), while red symbols represent birds that likely over-summered in the North Atlantic (n = 7). Grey and white alternating zones refer to months. Bird ID shown on the Y-axis of this figure, correspond with Bird ID of Figure 6.3.

Sanderlings was estimated to last 18.6 days. The mean departure date from the Arctic was estimated as 13 July. This coincides with the mean hatching date in northeast Greenland (13 July). The majority of clutches fails due to depredation (Reneerkens et al. 2014) and Sanderlings often leave their partner with the care of eggs (Reneerkens et al. 2011). When clutches are incubated by two adults, one of the partners always leaves the other parent with the chicks, as soon as they hatch (Reneerkens et al. 2014). This would explain the early departures from the Arctic tundra by the majority of assayed birds. The seven individuals that seemed to have over-summered in the North Atlantic were most likely second calendar year birds (Summers, Underhill & Prŷs-Jones 1995). The proportion over-summering Sanderlings in the North Atlantic (12%) is comparable to an earlier study by Lemke, Bowler and Reneerkens (2012) who estimated the percentage of juveniles in a wintering population in Scotland to be 6 – 9%.

At time of our isotope analyses, it was not common practice to use lipid-free tissues. It is clear now that lipids may influence isotopic values substantially, also in blood tissue (e.g. Rode et al. 2016). Specifically, high lipid contents in tissue biases $\delta^{13}C$ values downwards, while lipid contents may vary between individual and tissue type. Although our case study with Sanderlings clearly demonstrates the applicability of the double diet switch model, the estimated migration schedule may be biased for not using lipid-free tissues. To explore this possible bias, we corrected for lipid contents following the method of Post et al. (2007), who suggested to use C:N ratios of the sampled tissue to correct for lipid contents by adding a correction term to the estimated $\delta^{13}C$ values, and we reran the model with the 'lipid-free' approximate $\delta^{13}C$ values (of all tissues, from Sanderlings and prey). Using the 'lipid-free' data, the model did not converge for 14 birds (while all 59 birds converged when using incorrected values), indicating that corrections were inconsistent with the model. Using the approximated 'lipid-free' data of the remaining birds, resulted in an estimated departure date from the Arctic that was later than when using uncorrected data (24 July [CI 20 – 26 July], rather than 13 July), a shorter estimated staging duration (10.1 days [CI 7.6 – 14.9], rather than 18.6 days), but a similar arrival date in the Wadden Sea (31 July [29 July – 2 August] compared with 1 August) (n = 45). The model estimates using the 'lipid-free' data matched better with our expectations on the timing of southward Sanderling migration.

As it is likely that Sanderlings show moderate intraspecific variation, we used distributions of the input parameters rather than the mean values, for two reasons. First, individual dietary preferences cause stable isotopic values to vary slightly among individuals. Moreover, the discrimination factor that may be used to distinguish between diet and consumer may vary between individuals as well (supporting information I; Caut, Angulo & Courchamp 2009). Second, intraspecific variation in turnover rates is rather large and poorly understood (Martínez del Rio et al. 2009; Hahn et al. 2012). More accurate information about intraspecific variation in turnover rates is needed

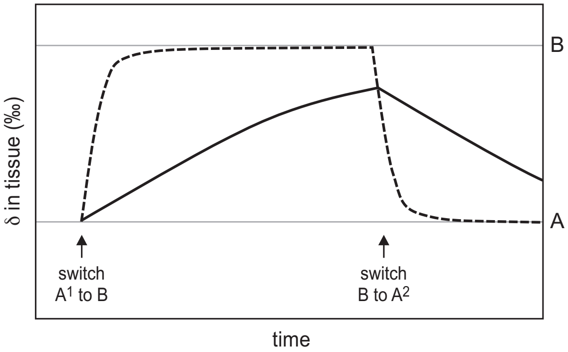


Figure 6.5: A special case of the double diet switch model, the ABBA-switch. This is a simplified representation of a 'switch-switching back' situation, from diet A¹ to B and from B back to A² describing how the isotopic values of two tissues, one with a fast turnover rate (striped black line) and a slow turnover rate (solid black line) , adapt from diet A to(wards) diet B back to(wards) diet A. The two grey lines (line A and B) represent the isotopic signature of the tissue in equilibrium with the two diets. The two arrows indicate the time of the two diet switches.

for more accurate estimations of individual seasonal scheduling. As the conditions for using the 'double diet switch model' can be met rather easily on the basis of a single time point stable isotope measurement of the target species (Table 6.1), the 'double diet switch model' allows a relatively simple way to assess seasonal schedules.

We encourage future use of our model for estimation of seasonal schedules of animals and emphasize that other isotopes than carbon can also be used (e.g. nitrogen or sulphur). The 'double diet switch model' might be particularly interesting in deciphering the timing and occurrence of migration in other migratory animals, animals with changes in food availability during a season (e.g. an animal that follows the food peak of different prey species), or in the timing of ontogenetic development of animals (e.g. from egg to juvenile to adult). Although not tested here, the 'double diet switch model' might not be limited to studies with switches between three isotopic levels, i.e. with diet switches from diet A to B to C, but might also be applicable to scenarios where the second switch reverses to the initial isotopic level, so a double diet switches from diet A^1 to B and from B back to A^2. We call this an 'ABBA switch' (see Figure 6.5). An ABBA switch may occur under temporary changing conditions such as e.g. breeding, drought, frozen foraging surfaces (no access to regular food) or injuries of the animal that restricts regular prey consumption. The ABBA switch could, theoretically, be studied with the regular formula of the 'double diet switch model' (see equation 2), where diet A^2 can be interpreted in the model as diet C. The model is thus generally applicable, and can be adapted to a wide range of taxa and situations in which animals use two or three distinct diets within a short period of time.

Acknowledgements

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Data accessibility

Data is deposited in the Dryad repository http://dx.doi.org/10.5061/dryad.t72b0.

Supporting information I:

Stable isotope values in equilibrium with three diets

General

In order to use the 'double diet switch model', the stable isotopic signatures of two tissues in equilibrium with the three diets (δ_A, δ_B, and δ_C) need to be known. These can be derived either directly by isotopic signals of the consumer or indirectly with help of the isotopic signals of the diet and a discrimination factors (DiF) by using the following formula; $\delta_{consumer} = \delta_{avg.(diet)} + DiF$, where $\delta_{consumer}$ is the isotopic signal of the consumer and $\delta_{avg.(diet)}$ is the isotopic signal of the average diet of the consumer.

A general *DiF* for $\delta^{13}C$ and $\delta^{15}N$ for mammals, fish, birds and insects is given in Caut, Angulo & Courchamp (2009). Note that the *DiF* is distinct for different tissue types.

Case study: sanderling migration

Here we show how we obtained the isotopic values for Sanderlings in equilibrium with their three diets along migration. For more information about the measurements, we refer to the main Methods.

Diet A – The $\delta^{13}C$ values of plasma and RBC of Sanderlings in equilibrium with their diet of arthropods in the Arctic breeding area were obtained directly by catching ten adult Sanderlings on their nest in Greenland (Zackenberg, 74°30'N, 21°00'W) in the second half of June 2009 (Table 6.2). For a description of the measurement procedure of $\delta^{13}C$ in plasma and RBC, see method section of main article.

Diet B – Southwest Icelandic coasts are important staging locations for Sanderlings using the East Atlantic flyway (Gudmundsson & Lindström 1992) where they feed on marine invertebrates along the shoreline (Reneerkens, Benhoussa, Boland et al. 2009; pers. com. Reneerkens & Hallgrímsson). The $\delta^{13}C$ signal of the diet of Sanderlings was determined from taking the mean of 25 prey items of five different invertebrate species (five per species) collected along the shoreline of Sandgerði (64,2 °N; -22,7 °E). All prey samples were put in separate vials, shortly stored at −20°C, freeze-dried in Iceland (Faculty of Life and Environmental Sciences, Reykjavik, University of Iceland) and transported to the Royal Netherlands Institute for Sea Research on Texel, The Netherlands, where stable isotope analyses were performed (see Method section of main article).

The mean $\delta^{13}C$ of the staging diet of Sanderlings was −18.21 ±1.19 ‰ (mean ± stdev, n = 25) , when foraging on a diet of *Apohyale prevostii* (-18.56±0.53), larvae of *Coelopa frigita* (−18.88 ±0.38), *Heteromastus filiformis* (−16.79 ±0.69 ‰), *Oligochaeta* sp, (−19.70 ±0.16 ‰) and *Idotea granulosa* (−17.13 ±0.28 ‰). To achieve a $\delta^{13}C$ signal for Sanderlings in equilibrium with the diet of the staging location we added a general discrimination factor for birds to the $\delta^{13}C$ signal of the diet, as described in Caut et al. (2009) ($\delta^{13}C$ DiF $_{plasma}$ = −0,078 ‰; $\delta^{13}C$ DiF $_{RBC}$ = 0,588 ‰).

Beside this indirect calculation, we measured the $\delta^{13}C$ signal in plasma and RBC of four Sanderlings caught in the Wadden Sea but with a $\delta^{13}C$ signal of plasma and RBC that represented the staging location (Table 6.2 of article, see Methods of article for measurement procedure).

Diet C – The $\delta^{13}C$ signal of Sanderling blood in equilibrium with the Wadden Sea diet was determined both directly and indirectly. Sanderlings in the Wadden Sea are mainly foraging on small Common Shrimp *Crangon crangon*, in late summer and much less often on other benthic organisms (Loonstra, Piersma & Reneerkens 2016). The Wadden Sea prey were collected in the Western Dutch Wadden Sea (near the islands Griend, Vlieland and Terschelling) close to where Sanderlings were caught for blood sampling. Samples were stored in separate vials at −20°C and processed at the NIOZ (see Methods). The mean $\delta^{13}C$ signal of the Wadden Sea diet of Sanderlings is −14,49 ±0,41 ‰ (mean ± stdev) when foraging on a diet of 90% *C. crangon* and 10% *Gammarus* sp ($\delta^{13}C_{\textit{Crangon crangon}}$ = −14,38 ‰ ± 0,42; n = 17; length = 20,78 ±4,96 mm and $\delta^{13}C_{\textit{Gammarus}\ \text{sp.}}$ = −15,43 ±0,39 ‰; n = 3; length = 15,02 ±2,28 mm). To achieve a $\delta^{13}C$ signal for Sanderlings in equilibrium with the diet of the Wadden Sea we added a general discrimination factor for birds ($\delta^{13}C$ DiF $_{plasma}$ = −0,078 ‰; $\delta^{13}C$ DiF $_{RBC}$ = 0,588 ‰) to the $\delta^{13}C$ signal of the diet, as described in (Caut et al. (2009).

In addition to this indirect calculation, we directly measured the $\delta^{13}C$ signal of Sanderlings in equilibrium with the Wadden Sea diet (Table 6.2 of article, see Methods for measurement procedure). Birds caught in the Wadden Sea were considered to be in equilibrium with their new diet if the $\delta^{13}C$ signal of plasma minus $\delta^{13}C$ signal of RBC was less than 0,23 ‰.

Verification of adequate discrimination factor – Indirect estimation of the $\delta^{13}C$ signal in plasma and RBC of Sanderlings is valid, since the outcome does not significantly differ from direct measurements (Table 6.2 of article). Especially the matching outcomes found in Wadden Sea are valuable, since this measurement was truly directly measured. Although the 'direct' measurement of the staging area was achieved by measuring birds in the Wadden Sea with an isotopic signature that represented the staging area, it matched the 'indirect' calculation of the stable isotopic signature in equilibrium with the staging area well (Table 6.2 of article).

Supporting information II:

Turnover rate estimation of carbon isotopes in Sanderling blood

Turnover rates of stable carbon isotopes in plasma and red blood cells (RBC) of Sanderlings are not available from the literature. Determining the turnover rates would require an intensive indoor bird experiment, which was unfortunately not possible. Instead, we used interspecific data on turnover rates in birds to determine plausible ranges of tissue-specific turnover rates in sanderlings. We estimated mean turnover rates from species-level relationships between body mass and turnover rate, and we used within-species variability, averaged over several species, to estimate the between-individual variation in turnover rates.

We used table S3 in Hahn et al. (2012), which contains data on body mass and ^{13}C half-life times for plasma and red blood cells (RBC) in 11 bird species and added a new reference by Doll, Lanctot, Stricker et al. (2015). When multiple studies on the same species were presented, we calculated averages per species. We searched the original sources for standard deviations (SD) of the half-life times or turnover rates, or we converted confidence intervals (CI) to standard deviations according to the formula

$$\mathrm{SD} = \frac{\mathrm{CI}}{2t_{\mathrm{n}-3,0.975}}. \tag{S1}$$

Here $t_{\mathrm{n}-3,0.975}$ refers the 97.5 percentile of a t-distribution with $n-3$ degrees of freedom because turnover rates are estimated by fitting a curve with 3 parameters.

Turnover rates (λ) and half-life times (HL) are related by $\lambda = \ln 2/HL$, but due to this nonlinear relationship, means and standard deviations of HL do not convert to the corresponding statistics for λ by direct substitution. Instead, we converted means and standard deviations in half-life times into the corresponding quantities for turnover rates according to second-order approximations which we derived using the delta-method:

$$\begin{aligned} \bar{\lambda} &\approx \frac{\ln 2}{\overline{HL}}\ 1 + \frac{\mathrm{SD}_{HL}^2}{\overline{HL}^2} \\ \mathrm{SD}_\lambda &\approx \ln 2\, \frac{\mathrm{SD}_{HL}}{\overline{HL}^2} \sqrt{1 + \frac{\mathrm{SD}_{HL}^2}{\overline{HL}^2}} \end{aligned} \tag{S2}$$

When HL SD's were not available, we used the average SD of the species if they were available. Finally, we calculated SD's for $\ln \lambda$, assuming that λ is log-normally distributed:

$$\mathrm{SD}_{\ln\lambda} \approx \sqrt{\ln 1 + \frac{\mathrm{SD}_\lambda^2}{\bar{\lambda}^2}} \tag{S3}$$

The results are presented in table S1. We used the median values of the SD's of $\ln \lambda$ in our sensitivity analyses to draw random combinations of $\ln \lambda_{RBC}$ and $\ln \lambda_{plasm}$a from a bivariate normal distribution.

We estimated allometric regressions of the form $y = ax^b$ to predict the mean $\ln \lambda$ for the average Sanderling body mass as sampled by us (68g), assuming that $\ln \lambda$ is normally distributed:

$$\ln \lambda = \ln a + b \ln x + \varepsilon \qquad \text{(S4)}$$

The results are shown in figure S1. We combined the thusly predicted values for Sanderlings with the median standard deviations of $\ln \lambda$ to obtain a plausible range of $\ln \lambda$ values for the sensitivity analyses:

$$\begin{aligned} \ln \lambda_{RBC} &= -2.91 \pm 0.22 \text{ (mean } \pm \text{SD)} \\ \ln \lambda_{plasma} &= -1.20 \pm 0.11 \text{ (mean } \pm \text{SD)} \end{aligned} \qquad \text{(S5)}$$

Table S1: Species-specific body mass [g], ^{13}C half-life times (H-L [day]) and turnover rates (λ [1/day]). Numbers with an asterisk are approximations based on half-life times. Bold numbers were used in the sensitivity analyses. Extended from table S3 in Hahn, Hoye, Korthals et al. (2012), with an additional reference of Dunlin turnover rates by Doll et al. (2015). YW=Yellow-rumped Warbler, ZF= Zebra Finch, GW= Garden Warbler, HS= House Sparrow, DU=Dunlin, RK= Red Knot, JQ=Japanese Quail, AC= American Crow, Ma= Mallard, GS=Great Skua and Ca= Canvasback.

Species	Mass	H-L RBC		H-L plasma		λ RBC		λ plasma		$\ln \lambda$ R.	$\ln \lambda$ p.
		mean	SD	mean	SD	mean	SD	mean	SD	SD	SD
YW	13	8.0	2.1	0.8	0.3	*0.093	*0.023	*1.09	*0.44	*0.25	*0.39
ZF	16	13.4				*0.053					
GW	20	5.4	0.8			*0.131	*0.018			*0.14	
HS	23	17.6	2.1	3.3		*0.040	*0.005	*0.21		*0.12	
DU	56	11.4	0.8			*0.061	*0.004			*0.07	
RK	148	15.1		6.0		0.046	0.003	0.11	0.03	0.07	0.24
JQ	190	11.4				0.062	0.006	0.27	0.04	0.10	0.14
AC	416	29.8		2.9		*0.023		*0.25			
Ma	980	31.9		4.3		0.022	0.007	0.16	0.03	0.31	0.66
GS	1220	15.7	1.0			*0.044	*0.003			*0.06	
Ca	1248	22.8				*0.031					
									median:	**0.11**	**0.22**

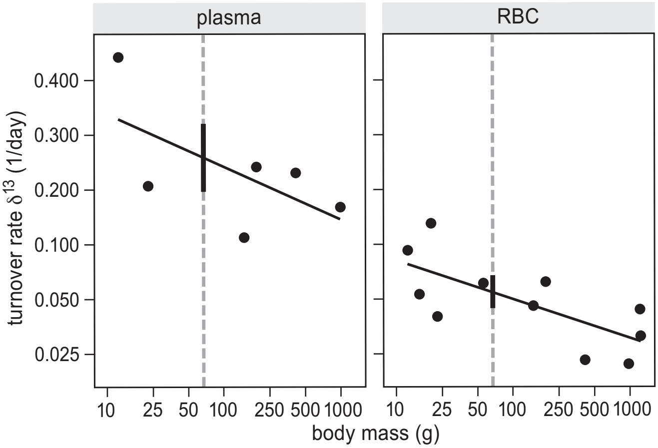
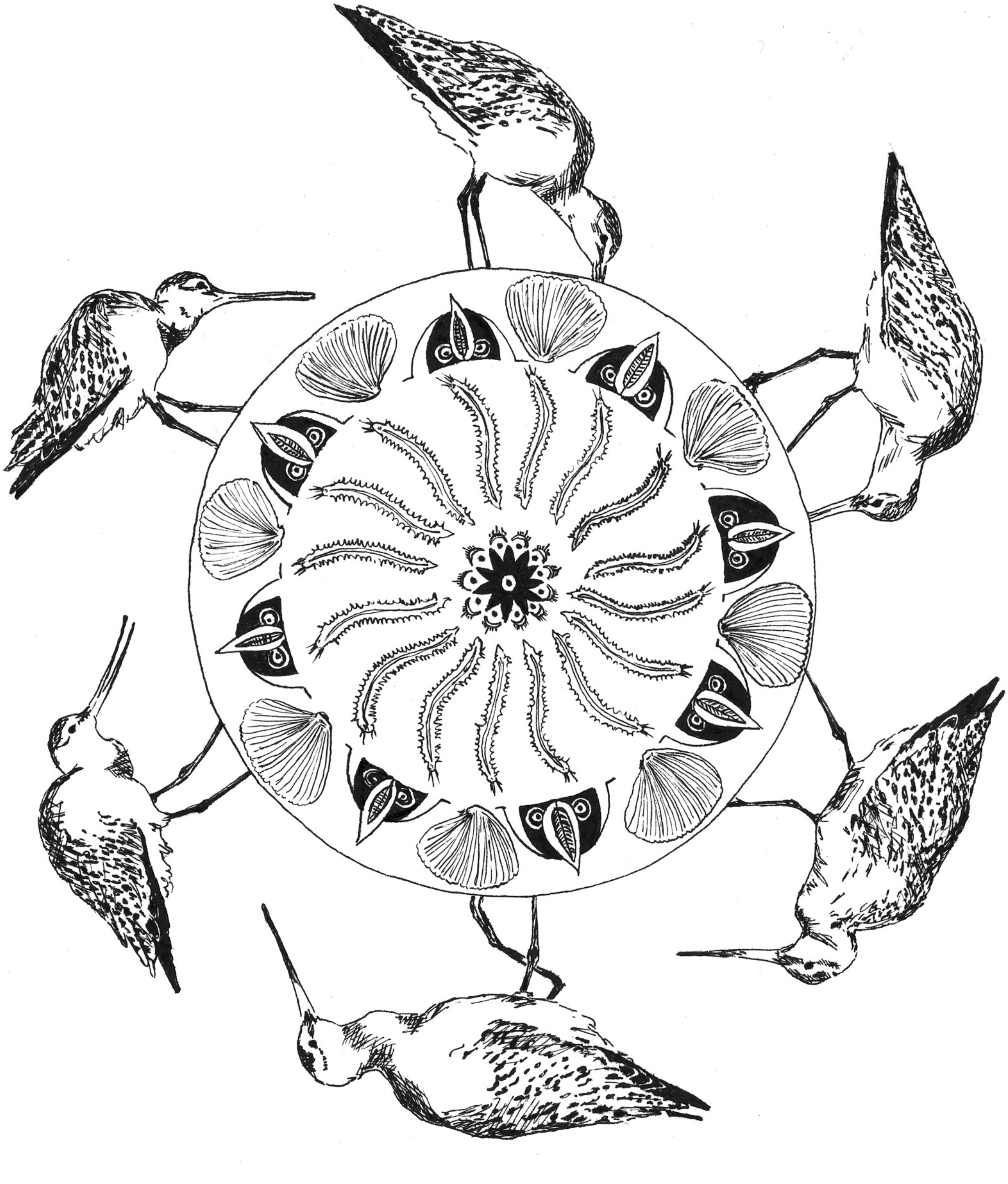


Figure S1: Allometric regressions for the turnover rate (λ) of δ^{13}C in blood plasma and red blood cells (RBC) vs. body mass in birds. Plasma: $\ln \lambda = 0.03 - 0.29 \ln BM$; RBC: $\ln \lambda = -2.00 - 0.21 \ln BM$. Dashed lines mark average body mass of Sanderlings (68 g). Solid vertical bars indicate average intraspecific variation (mean ±SD) in turnover rates.



CHAPTER 7

General discussion

Jeltje Jouta

In this thesis I made an attempt to characterize, with the help of the tell-tale stable isotopes, the food web of the Dutch Wadden Sea. I aimed at making this characterization *spatially* explicit as possible. With an eye to current restoration attempts and changes in the future, I also identified indicator species to document *temporal* changes in the food web. This allowed me to show why scale is such a crucial variable in food web studies. It also became clear to me that the finding and proper working definition of one or more indicator species for changing food web structures (over time and space) requires more work than could be addressed in a single PhD project.

In this final chapter I will briefly summarize the previous chapters, followed up by a synthesis of what is required to characterize food webs. In doing so, I will invoke the help of yet unpublished data which show the importance of spatial aspects. I will also suggest some potential indicator species to help trophically characterize the Wadden Sea ecosystem over time and space. Last but not least, I will compare an isotope-based food web structure of the Wadden Sea to four other, previously characterized intertidal ecosystems along the East-Atlantic flyway.

Complications in the use of stable isotopes for determining trophic positions

During the *Waddensleutels* project of which this thesis is part, we discovered that the Wadden Sea food web is a benthic-driven system, i.e. a system that is highly dependent on the local primary production on the mudflats of the intertidal zone (Chapter 3). Interestingly, the younger salt marshes of the Wadden Sea ecosystem are highly dependent on external inputs of nutrients from their neighbouring marine system, i.e. organic matter washed ashore and the guano of roosting birds (Chapter 2). The contrast between the younger saltmarsh and the intertidal systems signals the issue of relative openness versus closeness in relation to spatial ‘grain’. A food web with species with strongly varying levels of mobility is hard to characterize because of the spatially heterogeneous baselines – consumers will have been feeding across a daily home range on a variety of resources that can encompass a range of $\delta^{15}N$ baseline levels (Chapter 4). But not for all species this blurs the trophic signals, also not for highly mobile species. Scaled up to the entire Dutch Wadden Sea, spoonbills nevertheless showed tell-tale information (Chapter 5). This led to the conclusion that their dependence on relatively rare young demersal fish (rather than the more common shrimp) was much greater than we realized. This diet preference may explain why spoonbills breeding on the vast predator-free saltmarshes of the Wadden Sea islands reach carrying capacity so quickly (Oudman et al. 2017). This may be limited by food availability instead of breeding area. Spoonbills thus could serve as an indicator species, indicative for the density of young demersal (flat)fish in the shallow intertidal Wadden Sea, an food web variable of present concern due to low overall fish stocks in the ecosystem.

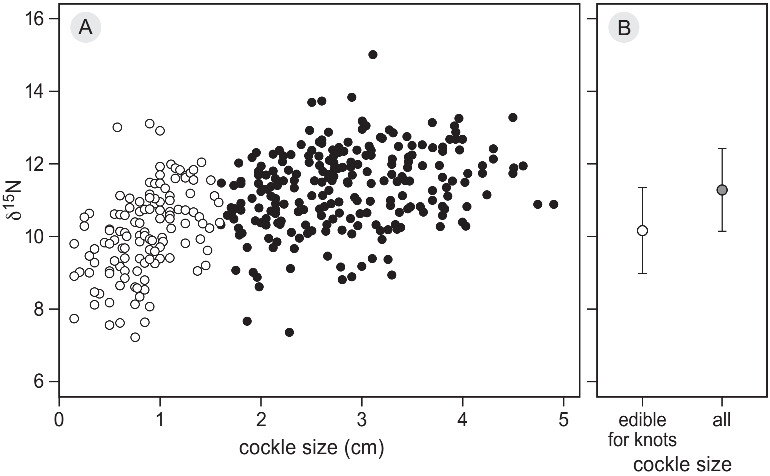


Figure 7.1: Trophic level, expressed as $\delta^{15}N$ (‰), in relation to prey size – the example of cockles *Cerastoderma edulis* (n = 376). Red knots feed only on small cockles of <16 mm (open circles) with a lower $\delta^{15}N$ value, compared to cockles larger than 16 mm (black circles) (graph A). Graph B shows that the $\delta^{15}N$ values (mean ± s.d.) of only edible cockle (<16 mm, open circles) is lower than all cockles sizes (grey circles), which can have significant effect for the justly estimation of trophic position of red knots.Knots can digest cockles of <16 mm, yet select 6.9 ±1.0 mm (Bijleveld et al. 2015) i.e. with lower $\delta^{15}N$ values, which will make the isotopic difference between preferred and mean cockles even steeper. Using a baseline based on cockles of all sizes would result in an overestimate of the trophic level too large. Collected in June-September 2011 and August-September 2013.

Also for other species stage-dependent diet preferences are key in understanding trophic positions, which can lead to complications in estimating trophic positions. An interesting example of this was revealed in my studies on edible cockles *Cerastoderma edule*, a bivalve consumed by several shorebird species. Small (and young) cockles have lower $\delta^{15}N$ values than larger (and older) cockles (Figure 7.1). This may result in a different isotopic signal between red knots who routinely eat only the smallest cockles as they have to ingest them whole (e.g. Bijleveld et al. 2015), versus, e.g., oystercatchers who forage on large cockles from which they excise the flesh (e.g. Sutherland 1982). If a constant food web baseline would be assumed for cockles that ignores this ontogenetic shift in stable isotope signal, this would overestimate the trophic position of oystercatchers versus red knots (Figure 7.1).

Once that the food source of a consumer has been established and corrections have been applied for ontogenetic shifts in isotopic composition, the estimation of food web positions is still holds complications. The turnover rates of different tissues (e.g. red blood cells, blood plasma, gonads, liver) may be very different, which gives the opportunity to explore seasonal diet and/or habitat shifts. Where the signal in high-turnover tissue indicates recent diet, the signal in slow-turnover tissues indicates the diet longer ago. We made use of this complication in our study on the estimation of migration scheduling of sanderlings (Chapter 6), It allowed us to devise a statistical method used the isotopic values of red blood cells and blood plasma (with reference to known turnover rates) to reconstruct diet histories.

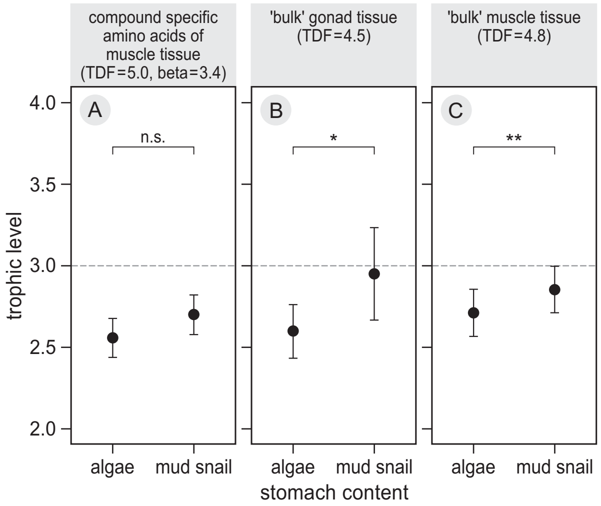


Figure 7.2: Trophic level (mean ± s.d.) of individual mullets *Chelon labrosus* showing a contrast in stomach content: either vegetable matter (diatoms (algae) and other components of biofilm) or mudsnail *Peringia ulvae* (itself a consumer of biofilm). In graph B and C the trophic level is of mullets was calculated with help of $\delta^{15}N$ values of muscle bulk or gonad bulk tissue and $\delta^{15}N$ values of the baseline (diatoms). This method of trophic level calculation requires solid information about the true underlying baseline, though the underlying baseline(s) of the target species can be difficult to determine (e.g. lack of knowledge about the food sources and spatial heterogeneity in baseline sources). Note that the trophic differentiation factor (TDF) between prey and predator may vary greatly between species, we here chose to use the TDF of muscle and liver tissue (here used for gonad tissue) given by Sacramento et al. (2015) since they studied the comparable fish species *Prochilodus lineatus*. Note that the actual TDF of Mullets might be slightly different, but the relative difference between mullets with a stomach content of algae or mud snails will not differ. Graph A shows the estimated trophic level of mullets with help of Compount Specific Isotope Analysis of Amino Acids (CSIA-AA) with $\delta^{15}N_{Glu\text{-}Phe}$ values ($\beta = 3.4$, TDF = 5.0) (Dale et al. 2011, Chikaraishi et al. 2010, 2014), this calculation only relies on the mullet tissue. CSIA-AA makes use of differences in the metabolic pathway of amino acids. Whereas the amino acid glutamate displays large enrichment of ^{15}N with trophic level, i.e. the trophic differentiation factor (TDF), the amino acid phenylalanine (Phe) remains unchanged with trophic level and thus reflects the base of the food web (Styring et al. 2010).

Another complication may arise if species forage on a mixture of resources, as shown by my study on the trophic position of grey mullet *Chelon labrosus*. This is a typical Wadden Sea fish that often behaves like a grazer of the biofilm (Cardona 2016), but may also eat mud snails (*Peringia ulva*) in large quantities. As mud snails themselves are grazers (on diatoms), one would predict mullets on a mud snail diet to be one trophic level higher than mullets eating algae. However, a mullet feeding on mud snails likely also ingests part of the algae containing biofilm and thereby, thus, may be expected to not be a full trophic level higher than mullets that eat only algae. When studied the trophic level of mullet in relation to their stomach content, this expectation was not sustained. (Figure 7.2), except possibly for the reconstructed trophic level based on $\delta^{15}N$ in the gonadal tissues (that represents the diet of some days ago and should therefore best represent the prey found in the stomach). Both, bulk analysis and Compound Specific Isotopic Analyses of Amino Acids (CSIA-AA) carried out on muscle tissue (explained in brief in the caption to Figure 7.2) suggest that the true, longer-term diets of mullets carrying either algae (and indeed other parts of the biofilm) or mud snails in their muscular gizzards at the time of capture is still rather mixed. This suggests that Mullets with different stomach contents (either algae or mud snails) do not reflect individuals with consistent diet preferences. Instead, the species seems to eat what is available on the top layer of the bottom of the sea.

The power of isotope analyses in food web studies

Having raised some caution about not bluntly using isotopic analysis, I now like to focus on power of this analysis. The isotope data for plasma in red knots hint at the possible use of stable isotopes as indicators of prey quality at the age or cohort level. Based on the studies of van Gils et al. (2005) we know that cockles, mud snails and Baltic tellins rank as prey of increasing quality for a forager that needs to crush and process the ingested shell material. The three prey isotopically (and usefully) rank along the $\delta^{13}C$ axis in a similar order (Figure 7.3). When I compared juveniles (birds that have arrived in the Wadden Sea for the first time 1–3 months earlier), 2nd calendar year post-summering birds and adult red knots *Calidris canutus*, I found that the older birds have higher ratios for both the C and the N isotopes. This strongly suggests that with age, size and experience they are able to eat prey of increasingly higher quality, at least in September 2011 and 2012 (see Bijleveld et al. 2016 for the capacity of red knots to make very fine distinctions using echo location in the sediment).

A final example of the power of stable isotopes in studies of diet and trophic position of single species in the food web is that of bar-tailed godwits *Limosa lapponica*, a species predominantly feeding on worms. Based on the blood plasma-based isotopic positions of the bar-tailed godwits and their two potential prey species, the ragworm

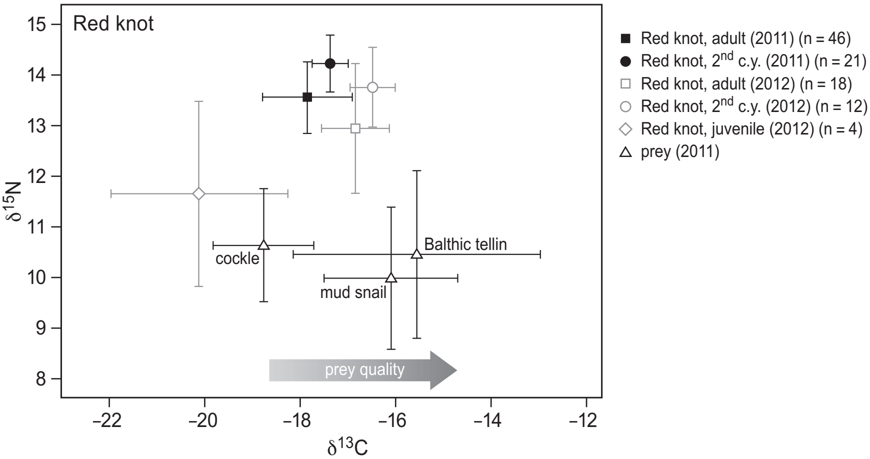


Figure 7.3: Trophic position (mean ± s.d.) of red knots (based on blood plasma) of different age categories in the August 2011 and from the end of July to mid October 2012 in relation to the position of their three potential prey (cockles *Cerastoderma edule* (n = 62 with size <16mm), mud snail *Peringia ulvae* (n = 38) and Baltic tellin *Macoma balthica* (n = 27)). Knots were caught with mist nets on De Richel (~53°17'50"N, 5°8'05"E), prey were collected in tidal basin Vlie in June-September 2011 during a spatially comprehensive monitoring campaign (Synoptic Intertidal Benthic Survey, SIBES).

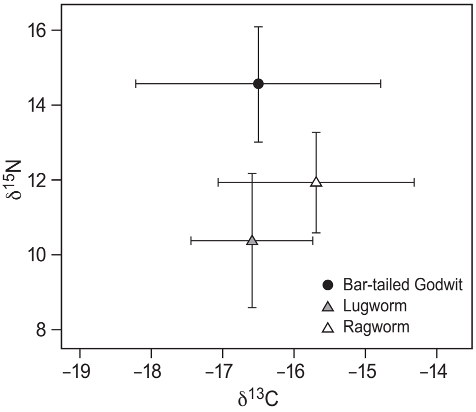


Figure 7.4: Trophic position (mean ± s.d.) of bar-tailed godwits (based on blood plasma, n = 13) in August-September 2011 in relation to the position of the two potential prey (ragworms *Hediste diversicolor* (n = 19) and lugworm *Arenaria marina* (n = 25)). Bar-tailed godwits were caught with mist nets on De Richel (~53°17'50"N, 5°8'05"E), prey were collected in tidal basin Vlie in June-July 2011 during a spatially comprehensive monitoring campaign (Synoptic Intertidal Benthic Survey, SIBES).

Hediste diversicolor and the lugworm *Arenicola marina* (Figure 7.4), it becomes clear that bar-tailed godwits captured at the De Richel high-tide roost in September 2011 were eating lugworms rather than ragworms. This nicely complements the more classic diet studies published by Duijns et al. (2013, 2014).

Food webs in space

Food webs are often presented as static representations of trophic interactions, but the species sets they describe are not static, not even under undisturbed conditions (Pimm, Lawton & Cohen 1991, Polis, Anderson & Holt 1997). To be most helpful to managers and politicians keen to do an evidence-based management of the Wadden Sea, the food web changes would be best described across space and over time. This should acknowledge that a food web is an interaction between species with widely different spatio-temporal habitat use. In reality, species are stepping in and out food webs over time and space, making them dynamic instead of static entities. However, these patterns may not be completely unpredictable. Species at the lowest trophic positions are often more sessile or have low mobility, while higher trophic organisms generally have a higher mobility (Figure 7.5). Moreover, some of the mobile higher trophic organisms even feed in adjoining ecosystems, inferring that the top of a food web is not necessarily totally dependent on the bottom of the represented food web. This includes spoonbills that forage mainly in the Wadden Sea but also use freshwater resources (Chapter 5). I thus suggest that – taking time and space and aging and mobility of species into account – food relations in an ecosystem may be more complex than can be represented in a single food web.

This dynamic view contrasts with classic visual representations of food webs, with producers on the bottom and top-predators at the top, typically reflecting a single part of an ecosystem at a relatively short period. This visualization thus implicitly assumes that the players are all residents, and that they are more or less dependent on each other within the confines of a relatively uniform ecosystem. This not being true, especially when migrants are involved, caused me to increasingly realize how many hidden assumptions such images of food web systems really have. Not all primary producers play an important role in the food web based on them, neither are higher trophic organisms (always) totally dependent on those specific basal resources. Indeed, in some representations the pyramid shape could be caused by the lower trophic levels being less mobile and thus more variable in space than the higher and more mobile species where averaging across space is inherent to their biology (Figure 7.5). Similar ideas where species at higher trophic levels are more mobile, hence coupling the dynamics of different food webs across space can be works of McCann and colleagues (McCann et al 2005, Rooney et al 2008) following up on the work of Polis and colleagues (Polis et al 1995).

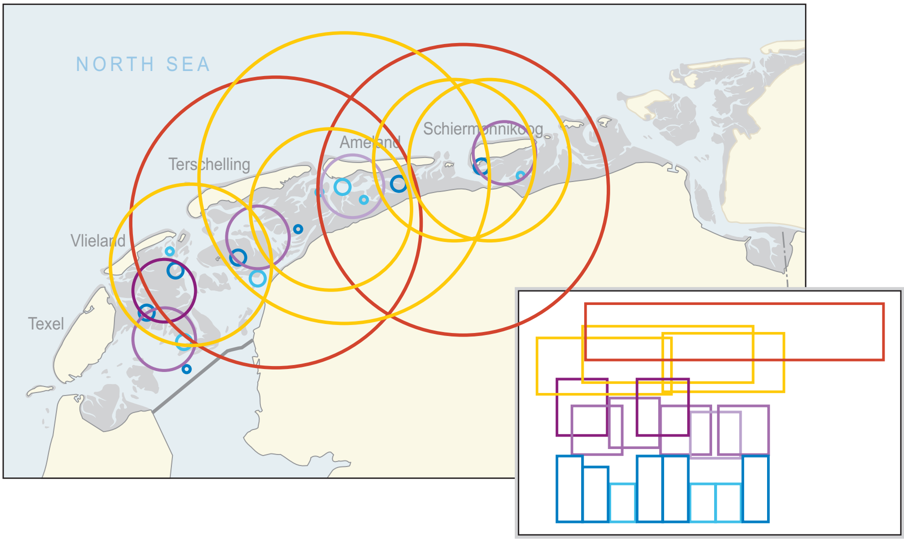


Figure 7.5: Simplified scheme of the consequences of variable home range size for visualizing a food web structure (in this case the Wadden Sea). The higher the mobility, the more mixing of underlying food sources, this resulting in a more homogeneous trophic position – along space – of these mobile organisms. Thus, higher trophic organisms with higher mobility will be representing a 'larger' ecosystem than the less mobile species, which are thus likely to shown bigger variation in web position across space. In this figure trophic levels are indicated by colour (light blue representing a lower trophic position than dark blue) and the degree of mobility by dot size (lowest mobility being indicated by the smallest dots).

These authors address the important topic how to analyse networks of trophic interactions whilst acknowledging mobility. And, how should this be integrated with the present knowledge of spatial heterogeneous baselines and flexible diets For example, an isotopic difference in $\delta^{15}N$ between the two locations can mean that (1) the trophic positions are different or (2) the baseline $\delta^{15}N$ shows spatially similar differences. This necessitates that we need to know what baseline value should be chosen while analysing trophic positions on the basis of stable isotopes. Mobile species such as birds or big predatory fish which operate across large areas with more spatial heterogeneity will average this spatial variability in baseline values (Chapter 4) to much greater extent than organisms with low mobility. Thus, when calculating a food web position, one should account for the mobility of consumers and of the spatial heterogeneity in the underlying food web (chapter 4). I have to conclude that isotope-based reconstructions of whole food webs may be rather poor indicators of ecosystem health. Isotopes are useful to indicate particular ecosystem relationships rather than state qualities, such as spoonbill isotopes indicating a reliance on small fish (Chapter 5) rather than indicating the magnitude of the food pyramid (bigger pyramids indicating healthier systems, see Introduction).

Characterizing 'average' food webs along a shorebird flyway

These complexities in the analysis of food webs should be accounted for when aiming for conclusions on trophic structure and its conservation implications. At a next larger scale, isotopic analysis of trophic positions can be used to even compare food web structure of different intertidal ecosystems along the East Atlantic Flyway by Catry et al. (2016). This study included four intertidal areas in Europe and West-Africa, but did not include the Wadden Sea (Figure 7.6).

The isotopic position of shorebirds varies between the studied areas (Figure 7.7). In the Tagus estuary, Portugal, shorebirds have the highest $\delta^{15}N$ value. This does not necessarily indicate a high trophic position as all the basal resources also occupy high $\delta^{15}N$ values (Figure 7.8). The Banc d'Arguin – an area that is still relatively pristine in relation to human influences compared with the Tagus estuary and the Wadden Sea – is an 'outlier' in Figure 7.7, with birds occupying a relative high $\delta^{13}C$ (indicating that the underlying food web relies mostly on marine basal sources) and a low $\delta^{15}N$ (stable nitrogen isotopes can reveal information about trophic positioning). The high $\delta^{15}N$

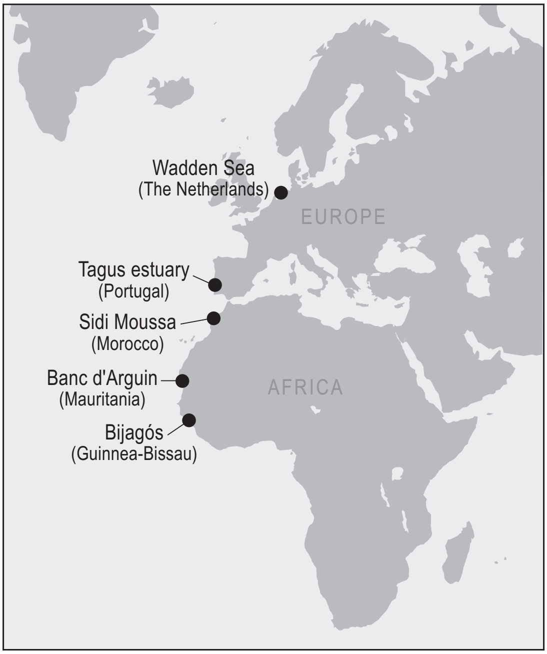


Figure 7.6: The five intertidal ecosystems along the East-Atlantic Flyway compared based on Catry et al. (2016) and my own measurements.

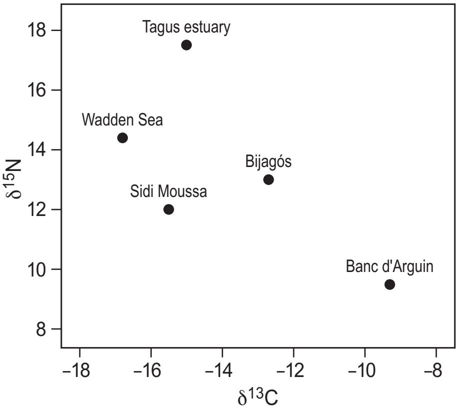


Figure 7.7: A comparison of the average positions of shorebirds in the $\delta^{13}C$ - $\delta^{15}N$ space of five intertidal ecosystems along the East-Atlantic Flyway. This is based on Catry et al. (2016) and my own measurements for the Wadden Sea, aligned with analyses of Catry as best as I could (see also Figure 7.8).

of the Tagus may show that this narrow estuary surrounded by intense agriculture receives the highest fertilizer loads (Gameiro et al 2007). The Wadden Sea occupies the opposite position from the Banc d'Arguin in the $\delta^{13}C$ - $\delta^{15}N$ space, with birds carrying a less marine signal at a high $\delta^{15}N$ value. So, on the basis of isotope positioning, the food webs of Banc d'Arguin and Wadden Sea seem to be very differently regulated intertidal ecosystems, less dependent on agricultural inputs, and with a less marine signal.

All intertidal systems along this flyway show high horizontal diversity in the food web (within trophic levels, as reflected by variation in $\delta^{13}C$ ratios), both for the primary producers as well as the consumers (Figure 7.8). This broad horizontal diversity indicates that the intertidal systems along the East Atlantic are built on a variety of basal organic sources (e.g. marine, benthic, brackish and freshwater sources). The consumers of the Bijagós Archipelago in Guinea-Bissau show the lowest horizontal diversity and do not seem to rely on mangrove-related biomass. The isotopic outlier on the bottom left in the two West-African sites may refer to the unique trophic position of the bivalves of the Lucinidae family (Figure 7.8). These bivalves host sulphide-oxidizing symbionts and thus have a mixotrophic diet (van der Geest et al. 2014); they depend on sulphite for their energy and on organic matter for their carbon and nitrogen source, that are likely consumed under highly anoxic conditions.

The $\delta^{15}N$ position of the shorebirds in these different ecosystems is in general ~4.5 ‰ above that of the primary producers (Figure 7.8). Only the position between birds and two of the basal sources (SOM and POM) in the Tagus estuary showed an enrichment of about ~7 ‰ $\delta^{15}N$. Since all primary producers and consumers in the Tagus showed enriched $\delta^{15}N$ values, the marine basal sources in the Tagus food web seem to be enriched in $\delta^{15}N$ indicative of nitrogen enrichment from inland sources.

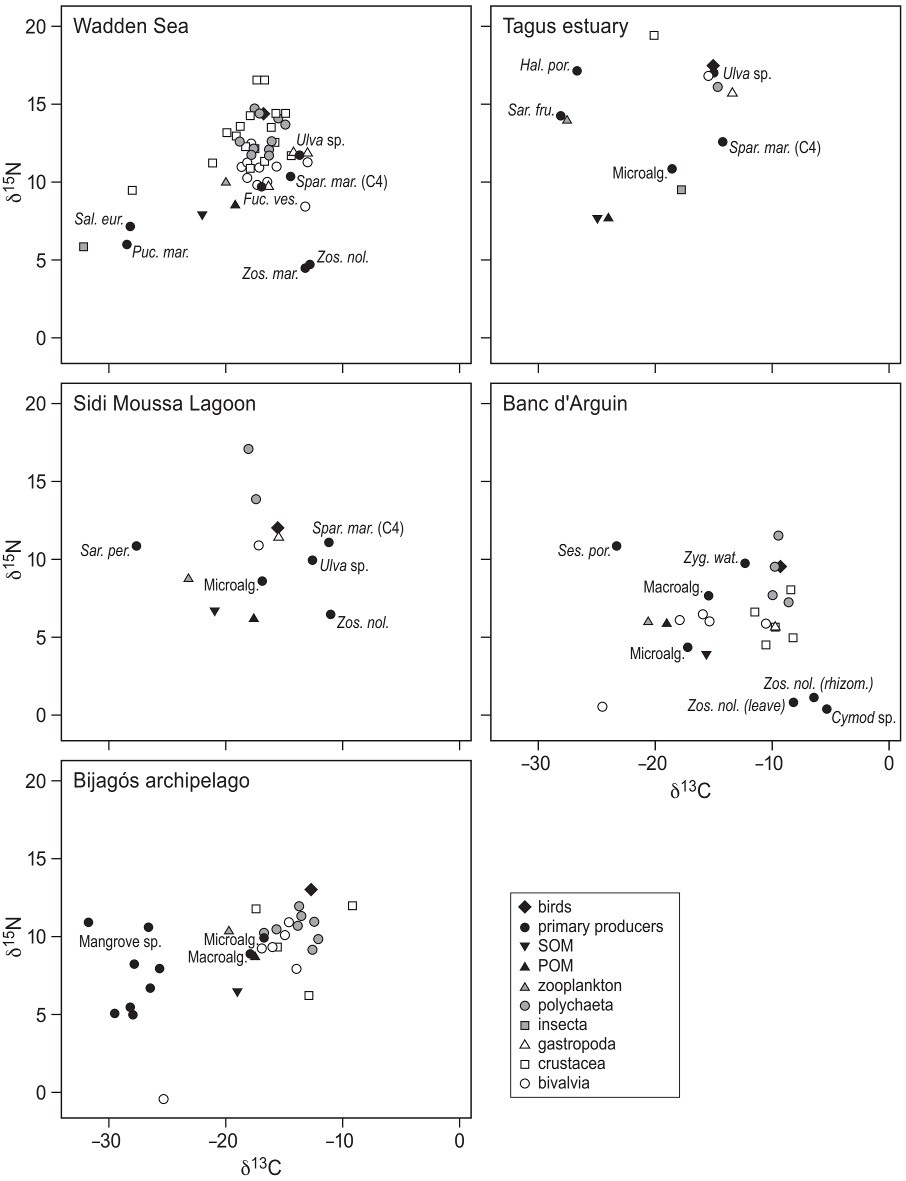


Figure 7.8: A comparison of the food webs in five intertidal ecosystems along the East-Atlantic Flyway in a δ^{13}C - δ^{15}N space. This is based on Catry et al. (2016) and my own measurements for the Wadden Sea.

An everlasting search for balance

In the introduction of this thesis (Chapter 1), I wondered why the heart of the Wadden Sea was still beating. The main character in the story *The tell-tale heart* by Edgar Allan Poe went mad and he wondered why the heart of the person he killed was still beating. I see similarities between this story and the thinking of today about natural wonders such as the Wadden Sea. Although the Wadden Sea ecosystem appears to be very flexible, the system has changed dramatically towards a likely impoverished state (to a mere 30% of the biological quality as found around the year 1700, according to Loske et al. 2003). We undeniably destroyed the unique brackish estuary of the Zuiderzee, embanked many salt marshes and estuaries in Friesland and Groningen, and called the remains the Wadden Sea. Meanwhile we cherish what is left as one of the still most natural ecosystems of the Netherlands (with the degree of enthusiasm varying greatly from person to person) and count on the resilience of this ecosystem to survive upcoming pressures such as climate change, without strong evidence that this trust is justified. Food web studies as outlined in this thesis can help in further unravelling the structure and functioning of this still extensive ecosystem, providing better information of how to protect its inspiring landscape, biodiversity and future resilience.

Supplemental information

Table S1: $\delta^{13}C$ and $\delta^{15}N$ signatures (mean ± SD) of basal food sources, producers and consumers (macroinvertebrates) collected the winter of 2012–2013 and/or 2013–214 in the Wadden Sea, Tagus estuary, Sidi Moussa, Banc d'Arguin and Bijagós archipelago. n = number of samples analysed (followed by the number of individuals included in each sample). Trophic guild is presented for the benthic macroinvertebrates. * = symbiosis with chemoautotrophic bacteria.

	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	n	Trophic guild
Wadden Sea, Netherlands				
Basal sources of organic matter				
POM	8.53 ±1.38	−19.21 ±1.81	73	
SOM	7.96 ±1.43	−22.00 ±3.03	103	
Algae				
Ulva sp.	11.75 ±2.71	−13.76 ±2.46	71	
Vegetation				
Spartina maritima (C4)	10.36	−14.45	1	
Zostera noltii (leaves)	4.49 ±2.91	−13.17 ±1.05	20	
Zostera marina (leaves)	4.73 ±1.42	−12.83 ±1.05	40	
Fucus vescilosus	9.68 ±3.22	−16.95 ±2.02	78	
Puccinellia maritima (salt marsh)	6.02 ±1.31	−28.43 ±0.90	20	
Salicornia europaea	7.18 ±0.77	−28.16 ±0.62	5	
Zooplankton	10.00 ±1.39	−20.00 ±2.33	63	
Bivalvia				
Scrobicularia plana	11.00 ±2.67	−15.67 ±1.79	22	Filter feeder
Abra tenuis	8.45 ±1.40	−13.21 ±2.47	18	
Abra alba	9.83 ±0.25	−17.30 ±0.18	2	
Cerastoderma edule	10.98 ±1.34	−18.65 ±1.30	399	
Crassostrea gigas	12.48 ±1.39	−17.84 ±0.86	37	
Mya arenarea	10.94 ±2.06	−17.15 ±3.45	47	
Mytilus edulis	11.25 ±1.52	−18.19 ±1.69	267	
Macoma balthica	11.29 ±2.34	−12.99 ±1.87	191	
Ensis directus	10.27 ±0.95	−18.17 ±1.53	56	
Tellina tenuis	10.01	−16.48	1	
Gastropoda & Polyplacophora*				
Peringia ulvae	9.74 ±1.72	−16.27 ±1.92	137	Detritivore
*Littorina littorea**	11.96 ±1.27	−14.22 ±1.43	60	
*Lepidochitona cinerea**	11.88 ±1.26	−13.01 ±2.77	19	
Polychaeta				
Hediste diversicolor	−16.06 ±1.70	12.63±1.69	167	Detritivore/Predator
Capitellidae n.id.				
-- *Heteromastus filiformis*	−17.67 ±1.32	12.16±1.09	20	
Nereidae n.id.				
-- *Alitta succinea*	−17.19 ±1.55	14.36 ±1.78	22	
-- *Allita virens*	−17.59 ±0.19	14.70 ±0.71	2	
Glyceridae n.id.				
-- *Glycera alba*	−18.82	12.60	1	
Nephtys sp.				
-- *Nephtys hombergii*	−14.96 ±0.99	13.69 ±1.15	25	
Lanice conchilega	−17.89 ±1.24	11.73 ±1.56	46	
Arenicola marina	−16.35 ±1.20	12.11 ±3.52	113	
Eteone longa	−15.57 ±1.15	14.10 ±1.61	40	
Scoloplos armiger	−16.35 ±1.30	11.75 ±1.35	79	

Table S1: Continued.

	δ^{15}N (‰)	δ^{13}C (‰)	n	Trophic guild
Wadden Sea, Netherlands				
Crustacea				
Crangon crangon	−14.98 ±1.69	14.41 ±1.90	363	Predator
Carcinus maenas	−15.77 ±1.30	14.42 ±1.64	1098	
Palaemon elegans	−17.32 ±2.09	16.55 ±1.96	70	
Ideotea linearis	−18.30 ±1.04	12.27 ±0.71	21	
Hemigrapsus takanoi	−16.10 ±1.29	13.53 ±1.37	25	
Hemigrapsus penicillatus	−17.67 ±1.32	12.16 ±1.09	20	
Liocarcinus holsatus	−17.94 ±1.46	14.29 ±1.28	106	
Bathyporeia sarsi/sp.	−14.48 ±1.53	11.70 ±1.92	8	
Corophium sp.	−17.97 ±2.32	10.88 ±2.99	19	
Gammarus locusta/sp.	−16.79 ±2.73	11.32 ±1.76	49	
Gastrosaccus spinifer	−19.90 ±0.87	13.17 ±0.94	15	
Hyperia galba	−19.18 ±0.91	12.97 ±1.69	21	
Palaemon serratus	−16.76 ±1.26	16.54 ±1.11	8	
Palaemon varians	−28.01 ±0.60	9.50 ±1.99	7	
Palaemon sp.	−21.12 ±5.14	11.25 ±2.36	34	
Praunus flexuosus	−18.82 ±4.73	13.60 ±1.58	94	
Urothoe poseidonis	−15.87 ±1.40	12.57 ±1.54	40	
Insecta				
Corixidae	−32.15 ±4.00	5.88 ±2.53	14	
Tejo estuary, Portugal				
Basal sources of organic matter				
POM	7.68 ±0.13	−24.00 ±1.41	3	
SOM	7.71 ±0.67	−24.95 ±1.21	3	
Algae				
Ulva sp.	17.02 ±2.80	−14.97 ±3.43	3 (5–10)	
Microalgae	10.87 ±1.27	−18.55 ±4.40	2	
Vegetation				
Spartina maritima	12.6	−14.22	1 (10)	
Sarcocornia fruticosa	14.28 ±2.42	−28.07 ±1.01	3 (10)	
Halimione portulacoides	17.20 ±2.34	−26.68 ±1.33	3 (10)	
Zooplankton	13.99 ±0.66	−27.55 ±3.96	2	
Bivalvia				
Scrobicularia plana	16.86 ±2.19	−15.38 ±1.74	11 (5–25)	Filter feeder
Gastropoda				
Hydrobia ulvae	15.73 ±1.73	−13.34 ±1.02	7 (>25)	Detritivore
Polychaeta				
Hediste diversicolor	16.14 ±2.21	−14.62 ±0.37	10 (4–10)	Detritivore/Predator
Crustacea				
Crangon crangon	19.47 ±0.95	−20.09 ±1.63	3 (5–10)	Predator
Insecta				
Chironomidae larvae	9.51 ±1.33	−17.73 ±0.29	3 (>20)	
Sidi Moussa, Morocco				
Basal sources of organic matter				
POM	6.14 ±2.74	−17.57 ±9.46	2	
SOM	6.71 ±0.49	−20.94 ±2.09	2	
Algae				
Ulva sp.	9.93 ±2.57	−12.55 ±5.37	2 (5–10)	
Microalgae	8.60 ±1.69	−16.83 ±3.27	2	

Table S1: Continued.

	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	n	Trophic guild
Sidi Moussa, Morocco				
Vegetation				
Spartina maritima	11.09 ±0.11	−11.12 ±3.85	2 (10)	
Sarcocornia perennis	10.85 ±4.07	−27.63 ±2.12	2 (10)	
Zostera noltii (leaves)	6.46 ±2.21	−10.97 ±6.28	2 (10)	
Zooplankton	8.74 ±2.09	−23.16 ±4.54	2	
Bivalvia				
Scrobicularia plana	10.88 ±4.66	−17.16 ±2.36	7 (5–10)	Filter feeder
Gastropoda				
Hydrobia ulvae	11.40 ±0.64	−15.43 ±1.45	6 (>25)	Detritivore
Polychaeta				
Hediste diversicolor	13.86 ±2.28	−17.38 ±1.20	7 (4–10)	Detritivore/Predator
Diopatra neapolitana	17.09 ±0.01	−18.05 ±0.15	3 (4–10)	Detritivore/Predator
Insecta				
Chironomidae larvae	4.39 ±0.16	−18.96 ±0.11	3 (>20)	
Banc d'Arguin, Mauritania				
Basal sources of organic matter				
POM	5.88 ±0.21	−19.02 ±5.85	2	
SOM	3.93 ±0.66	−15.58 ±3.28	2	
Algae				
Macroalgae n.id.	7.68 ±0.72	−15.38 ±1.38	2 (5–10)	
Microalgae	4.38 ±1.14	−17.19 ±3.18	3	
Vegetation				
Zygophyllum waterlotii	9.78 ±3.75	−12.31 ±3.75	2 (10)	
Sesuvium portulacastrum	10.90 ±6.61	−23.31 ±3.18	2 (10)	
Zostera noltii (leaves)	1.13 ±0.27	−6.42 ±1.81	2 (10)	
Zostera noltii (rhizomes)	0.82 ±0.72	−8.14 ±2.17	2 (10)	
Cymodocea sp. (leaves)	0.37 ±0.24	−5.30 ±4.60	2 (10)	
Zooplankton	5.97 ±0.22	−20.60 ±2.23	2	
Bivalvia				
Abra sp.	5.87 ±0.28	−10.52 ±2.82	6 (>45)	Filter feeder
Anadara senilis	6.11 ±0.05	−17.89 ±0.40	3 (5–10)	Filter feeder
Diplodonta diaphana	6.02 ±0.55	−15.35 ±1.35	6 (3–5)	Filter feeder
Dosinia isocardia	6.48 ±0.83	−15.88 ±1.54	7 (10–20)	Filter feeder
Loripes lucinalis	0.53 ±1.20	−24.50 ±1.02	12 (10–20)	Symbiont*
Gastropoda				
Hydrobia ulvae	5.66 ±0.76	−9.72 ±2.25	6 (>50)	Detritivore
Polychaeta				
Capitellidae n.id.	7.70 ±0.24	−9.93 ±0.73	6 (15–20)	Detritivore
Nereidae n.id.	7.25 ±0.74	−8.57 ±2.03	5 (7–20)	Detritivore/Predator
Glyceridae n.id.	9.54 ±0.58	−9.67 ±1.01	5 (2–5)	Detritivore/Predator
Crustacea				
Amphipoda n.id.	4.52 ±0.13	−10.51 ±1.70	5 (>30)	Detritivore
Uca tangeri	5.66 ±0.18	−9.68 ±0.30	3 (5)	Detritivore
Carcinus aestuarii	6.62	−11.45	1 (5)	Detritivore/Predator
Palaemon elegans	8.06 ±0.19	−8.40 ±0.33	3 (10)	Detritivore/Predator
Idotea chelipes	4.97 ±0.41	−8.17 ±0.19	2 (5–10)	Grazer

Table S1: Continued.

	δ^{15}N (‰)	δ^{13}C (‰)	n	Trophic guild
Bijagós, Guinea–Bissau				
Basal sources of organic matter				
POM	8.66	−17.55	1	
SOM	6.52 ±1.19	−19.03 ±0.7	2	
Algae				
Macroalgae n.id.	8.89	−17.90	1 (5)	
Microalgae	9.89	−16.69	1	
Vegetation (mangroves)				
Avicenia sp. (leaves)	7.96	−25.66	1 (10)	
Avicenia sp. (pneumatophores)	6.70	−26.46	1 (10)	
Conocarpus erectus (leaves)	10.58	−26.58	1 (10)	
Laguncularia sp. (leaves)	5.06	−29.47	1 (10)	
Laguncularia sp. (pneumatophores)	4.96	−27.93	1 (10)	
Rhizophora sp.(leaves)	5.45	−28.15	1 (10)	
Rhizophora sp. (pneumatophores)	10.92	−31.71	1 (10)	
Rhizophora sp. (roots)	8.23	−27.80	1 (10)	
Zooplankton	10.35	−19.73	1 (10)	
Bivalvia				
Arca sp.	10.07 ±0.15	−14.90 ±0.26	3 (5–10)	Filter feeder
Anadara senilis	10.90 ±0.10	−14.53 ±0.58	3 (5)	Filter feeder
Dosinia sp.	9.22 ±0.36	−16.91 ±0.46	3 (5–10)	Filter feeder
Tagelus adansoni	9.29 ±0.10	−15.93 ±0.23	3 (5–10)	Filter feeder
Tellinidae n.id.	7.90 ±0.62	−13.93 ±0.45	3 (5–10)	Filter feeder
Lucinidae n.id.	−0.45 ±0.21	−25.30 ±0.30	3 (5–10)	Symbiont*
Polychaeta				
Capitellidae n.id.	11.95 ±1.71	−13.70 ±1.11	6 (10–15)	Detritivore
Glyceridae n.id.	10.23 ±0.78	−16.71 ±1.34	2 (2–5)	Detritivore/Predator
Nephtys sp.	10.95 ±0.21	−12.41 ±0.57	2 (2–5)	Detritivore/Predator
Cirratulidae n.id.	10.66 ±0.15	−13.80 ±0.17	2 (5–10)	Detritivore
Maldanidae n.id.	9.15 ±0.07	−12.55 ±0.21	2 (2–5)	Detritivore
Spionidae n.id.	10.45 ±0.08	−15.62 ±0.19	2 (5–10)	Detritivore
Eucinidae n.id.	9.85 ±0.35	−12.05 ±0.64	2 (2–5)	Detritivore/Predator
Lumbrineris sp.	11.31 ±0.78	−13.50 ±0.85	2 (2–5)	Detritivore/Predator
Crustacea				
Axiidae	11.80 ±0.57	−17.40 ±0.14	2 (5–10)	Detritivore/Predator
Uca tangeri	6.23 ±0.29	−12.86 ±0.42	3 (2–5)	Detritivore
Grapcidae n.id.	9.32 ±0.48	−15.58 ±3.26	5 (2–5)	Detritivore
Dendrobranchiata n.id.	11.97 ±0.50	−9.13 ±0.15	3 (2–5)	Detritivore/Predator

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Biography

Jeltje Mooiman-Jouta is an ecologist, specialized in stable isotopes and ecosystems, with interdisciplinary skills in education and arts. Her doctoral studies she dedicated to the food web of the Wadden Sea.

She was born and raised in the countryside of Fryslân, The Netherlands, where she lived with her parents, brother and sister. She enjoyed a serene childhood, with a lot of outdoor playing and other free and unbounded creative activities. Already as a toddler she caught birds barehanded to admire them closely and spend hours making detailed drawings, skills that turned out to be useful in her adult life.

After high school she did her bachelor Biology, followed up by a master Ecology and master Education at the University of Groningen, with part of the master Scientific Illustration at the University of Maastricht and Maastricht Academy of Fine Arts as an intermezzo. She got the opportunity to work with a group of leading scientists during her PhD studies within project Waddensleutels, being supervised by Prof. Theunis Piersma and Prof. Han Olff at the NIOZ and University of Groningen.

Jeltje lives with her husband Maarten and their children Wout and Bregje, aged 4 and 3, in the forest landscape of Oranjewoud. While finishing her dissertation she worked as a biology and art teacher at a high school, where she aimed to trigger pupils' wonderment, enthusiasm and care for life. She's devoted to raising their children, loves to create things, is interested in and likes to contemplate the human way of living and its impact on nature and is passionate about the beauty of nature.

List of publications:

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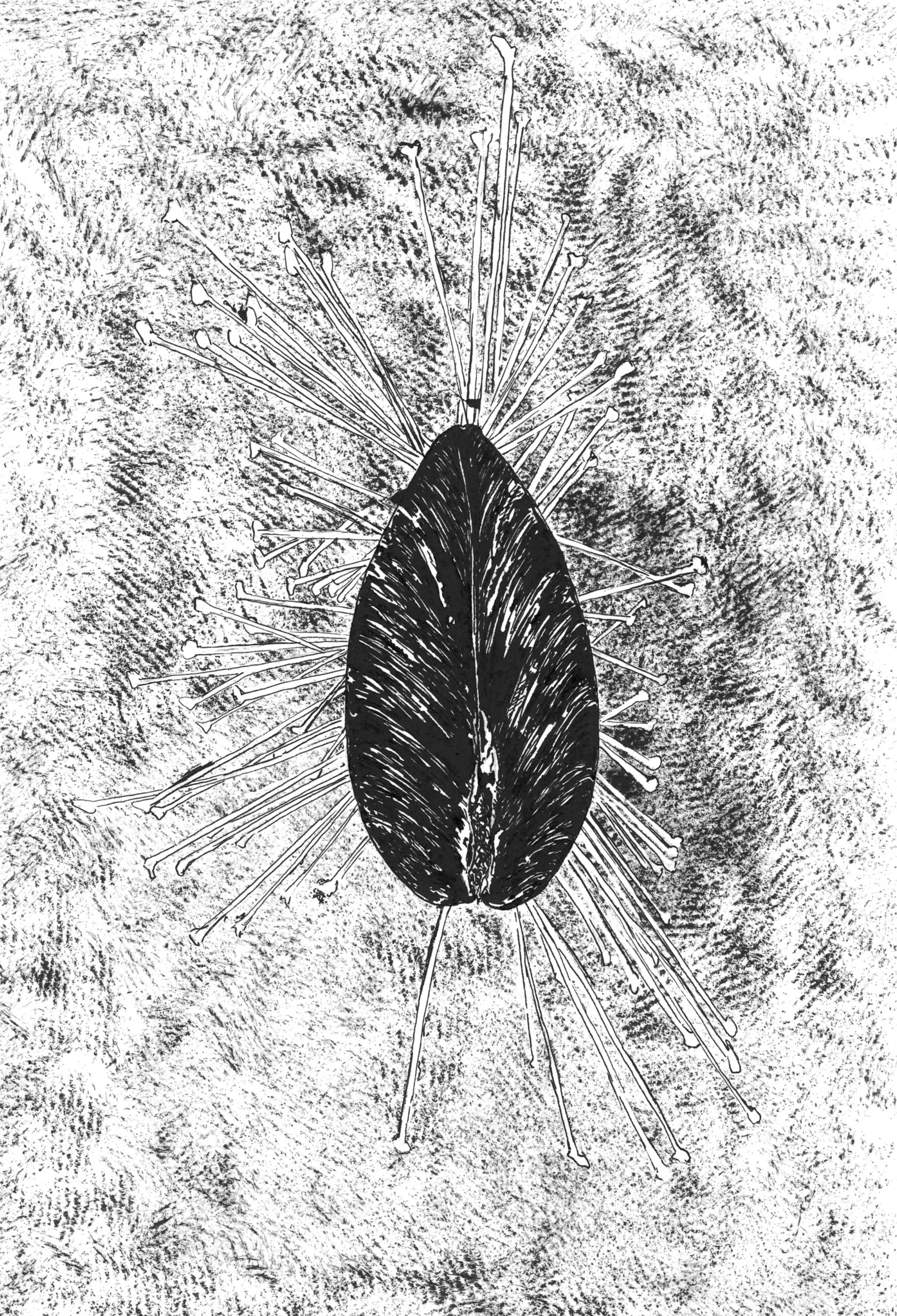


Summary

Introduction

Ecosystems are the basis of the world's ultimate life-support systems. Ecosystem health is crucial for the success and survival of humans. Ecosystems have a changeable nature and a stable ecosystem is not static, but face changes that can often be defied by the ecosystem. However, some bigger (human-driven) interference cannot be defied, causing ecosystem degradation. It is therefore valuable to understand the current state of the ecosystem and the consequences of potential disruptions. The analysis of food web structures has become a commonly accepted method to determine the state of ecosystems. A food web is a diagram depicting who eats whom in ecosystems. When assessed over time or space, food web structures can instruct us about the effects of disturbances and biodiversity loss, show the sensitivity of species and their connectance.

Reconstruction of food webs, with help of stable isotopes analysis, can be used as a means to study the state of an ecosystem. Stable isotopes of nitrogen and carbon are powerful and frequently used tools to reconstruct food webs. Stable isotopes are used to determine the food web position of species or species groups, making it possible to create a two-dimensional food web. The stable nitrogen isotope ($\delta^{15}N$) is used to elucidate the trophic structure, displayed on the vertical axis in a food web. The stable carbon isotope ($\delta^{13}C$) provides information on the input of carbon sources at the base of the food web and can thus infer the energy transfer through food webs, displayed on the horizontal axis in a food web (pyramid).

At first sight, the Wadden Sea might seem rather species-rich, but this is – at least relative to historical references – far from true. The last 1000 years, the Wadden Sea ecosystem last part of her food web complexity, often caused by human influences. Conservation and recovery became key priority for nature management in the Wadden Sea. Key processes that lead to and at the same time indicate the evolvement into a stable and healthy Wadden Sea ecosystem are thought to be: (1) recovery of the ecosystem engineering sea grass beds, intertidal blue mussel beds and even oyster beds who had a very important role for the total food web and (2) the re-appearance of top-predators. It is valuable to gain insight in the current state and recovery of the Wadden Sea ecosystem. Studying the (change of the) shape of the food web structure of the Wadden Sea can unravel the dependency of the system to changes in species(numbers) and dietary changes. A food web reconstruction of the Wadden Sea ecosystem can help predicting how the Wadden Sea will react to disturbances or restoration and is crucial for timely intervention.

This thesis describes the complexity of food webs, analysed mainly with help of stable isotopes. Who eats whom in an ecosystem, and can this be translated to a food web that integrates time and space? How can food web studies by valuable? I searched for the possibilities to use the food web position of indicator species as an indicator for the (spatially) variable state of the Wadden Sea food web.

Structure of the food web

In **Chapter 2** we show how a food web is formed and changes through time. This is studied by a reconstruction of the food web of the salt marsh of the Wadden Island Schiermonnikoog, with a focus on the role of extern nutrient input. Making use of a hundred year old salt marsh succession, we could study the food web from a zero to hundred year old salt marsh. We found that the young salt marsh is still very dependent of the input of extern nutrients from the adjacent intertidal Wadden Sea. On the young salt marsh, detritivores (organisms feeding on excreted and dead organic matter) are fed by the extern nutrient source, the Wadden Sea, and the detritivores are eaten by carnivores. Later in succession the food web becomes ‘classic’, with an intern nutrient cycle, where plants are eaten by herbivores, who are eaten by carnivores and where detritivores consume the all wasted matter and thereby make it a closed food web cycle. The (initial) structure of a food web therefore requires interaction of nutrients and energy flows between ecosystems.

In order to understand the marine Wadden Sea ecosystem it is valuable to know how autonomous this ecosystem is and by which nutrient sources it is fed, such as external sources or benthic or pelagic algae (resp. from outside the Wadden Sea ecosystem, on the bottom living marine algae and in the sea water floating algae). In **Chapter 3** we show that the Wadden Sea ecosystem is mainly internally fed by local benthic algae and that the above-lying food web is mainly dependent on this benthic energy. Besides, we found a high spatial heterogeneity in carbon isotope values of benthic algae. This emphasizes the importance of spatially sampling the benthic and pelagic algae, in order to create a reliable (base of the) food web. Detailed representations of the Wadden Sea food web are therefore difficult to represent.

Constraints of food web and isotope analysis

We found that, beside the spatial heterogeneous base of the food web, more complicating factors hinder us to provide a representative draft of the Wadden Sea food web. Being, changes through time, ontogeny, age and mobility of species, species with dependency of multiple ecosystems and the complexity of measuring methods. In Chapters 4, 6 and 7 we studied how a food web position can be estimated.

In **Chapter 4** we show that the base of the food web is also spatially heterogeneous for nitrogen isotopes and that the horizontal position of species in a food web are dependent of correctly estimating this (mean) base of the food web. Mobility and the underlying benthic or pelagic baseline(s) of a species define whether we can correctly estimate a trophic position. Whether spatial variation should be taken into account, when estimating the trophic position, varies between species depending on mobility and underlying benthic or pelagic baseline(s).

Also the ontogeny of an individual can be decisive for the food web position. For illustration, we see in **Chapter 7** that nitrogen isotope values increase with cockle size. Knowledge about the size of cockles that a cockle-consuming organism eats is therefore of influence for the estimation of a trophic position. Furthermore we show that a species can have a varying food web position, depending on its ontogenetic phase.

A correct measuring method will be crucial for the estimation of a correct food web position. (1) Individuals within a species can have different diets. (2) Different tissues of one individual give a diet description varying from the last days to the last months or years and thereby the isotope values of different tissues will likely vary. (3) The measuring process in the laboratory can also affect the isotope value and thus the estimation of a food web position. Do we measure the bulk sample, some lipids or only just a few amino acids? In **Chapter 7** we show with help of grey mullets how a trophic position is dependent on measuring methods. What measuring method is most suitable depends on the research question.

In **Chapter 6** we show that some species use the Wadden Sea ecosystem only temporarily. Sanderlings change diets while migrating. On arrival in the Wadden Sea they still contain tissue from former locations where they foraged along the migration route. This research shows that food webs of temporary (migrating) species in an ecosystem occupy a special position in a food web. They import external organic matter (feces), their measured isotopic values and diet can thus still show information from former foraging locations and their stay is merely temporarily. We simultaneously show how powerful isotope analysis can be, for by performing one isotope measurement (of two tissues) from one individual sampled at location C we were able to trace the migration schedule of this individual from location A to B to C.

Species in a food web are far from static, not even under undisturbed conditions. Therefore, we would really like to describe how food webs change over time and space. To a certain extent we can account for temporal and spatial variation, since higher trophic organisms often have a higher life expectancy and a higher mobility, species higher in the food web will partly equalize the differences on a smaller scale. Though sessile species, who occur mostly at the bottom of the food web, do not level-out differences and here profound differences in food web position may exist through time and space. In order to analyse the nuanced processes that play a role in disturbances or recovery, a detailed view of the ecosystem is requisite and a general description of the food web will be inadequate. My **conclusion** in the general discussion is therefore that the food relations in an ecosystem may be more complex than can be represented by a single food web.

Power of isotope analyses in food web studies

Yet, we can learn (and have learned a lot) from food web studies analysed with help of stable isotopes. Firstly, the use of indicator species can be significant for specific food relations in an ecosystem. Isotope measurements can give insight in misunderstood processes within part of the food web. In **Chapter 5** we show how spoonbills can function as indicator species for the state of the young (flat)fish in the shallow intertidal zone of the Wadden Sea. The last decennia spoonbills returned as a breeding bird to the Wadden islands. The circumstance on these islands seem rather ideal; with enough breeding areas, even increasing numbers of breeding colonies, very low degrees of predation and disturbance, seemingly unlimited extends of foraging area and even an increase in shrimp (the repeatedly reported main prey species). We studied the often stated assumption that shrimp are the main prey of spoonbills while breeding in the Wadden Sea, by analysing the diet of spoonbill chicks by regurgitate and isotope analyses. Both methods showed that not shrimp but small (flat)fish are the main prey during the breeding season. Unlike shrimp, small flatfish have been reported to be rather scarce in the Wadden Sea the recent years, which is likely the cause of the flattening spoonbill population and the decreasing chick survival in the Wadden Sea ecosystem. We predict that recovery of the young (flat)fish densities in the shallow parts of the Wadden Sea will be reflected in the diet of spoonbill chicks. Spoonbills can thus function as an indicator species for the (flat)fish stocks in the shallow intertidal Wadden Sea.

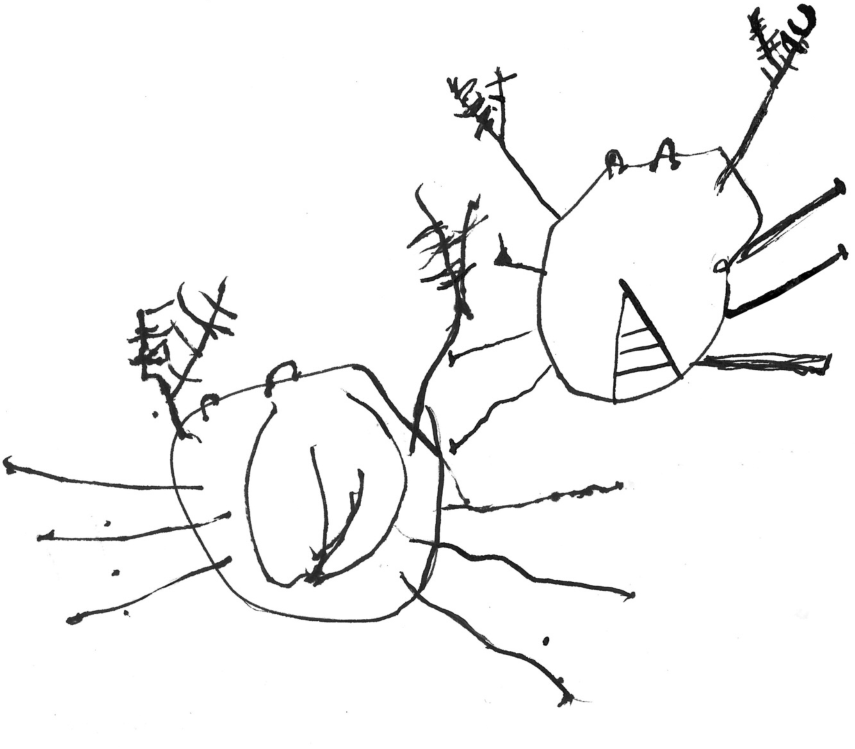
In **Chapter 7** the power of isotope analysis in food web studies is shown by a diet study of **bar-tailed godwits** and **red knots**. Isotope analyses prove an elegant method to determine which worm species is preferred by bar-tailed godwits. The prey quality of red knots appears to be made clear in a $\delta^{13}C\sim\delta^{15}N$ bi-plot with a sliding scale from left to right. We found that experienced red knots have a diet consisting of qualitatively higher prey species. It thus seems that researchers and nature managers can get insight in prey bivalve availability for knots (cockles, mud snails and Baltic tellin) by studying the carbon and nitrogen isotopes of red knot blood. Red knots could like a indicator species for the availability of high quality shellfish.

Secondly, a general food web can be used to compare ecosystems. In chapter 7 we compare five intertidal ecosystems in Europe and West-Africa along the East-Atlantic migration route. The intertidal areas the Wadden Sea and Banc d'Arguin seem to be differently regulated, with less agricultural influences and a less marine signal. The intertidal area of the Tagus estuary seems enriched by external inland nitrogen sources. Although the five intertidal areas show differences, there are certainly also similarities such as a broad base of primary sources (a wide $\delta^{13}C$ range).

Conclusion

In summary, it appears to be very difficult or impossible to represent a time and space integrated view of who-eats-whom in an ecosystem (a complete food web). This is because too many complicating factors play an important role in describing all food relations in one food web, such as; temporal change, mobility of species, differences within species (e.g. diet changes due to ontogeny and size), species who are dependent of multiple ecosystems and complexity of measuring methods. A food web can be valuable to study generalities, for example when comparing ecosystems. Furthermore, the study of individual food web positions (of indicator species) can serve as indicators for the variable state of the Wadden Sea food web.

I end my thesis with a somewhat philosophical and hopefully unfounded pessimistic view on what will happen with the small remains of the Zuiderzee, called the Wadden Sea. I cherish this piece of The Netherlands and admire her resilience, but fear the nonchalance with which man influences nature.



Samenvatting

Introductie

Ecosystemen liggen aan de basis van 's werelds ultieme levenskracht. De gezondheid van ecosystemen is cruciaal voor de overleving van mensen. Een ecosysteem heeft een veranderlijk karakter en een stabiel ecosysteem is dan ook niet statisch maar wordt voortdurend blootgesteld aan stressfactoren die het ecosysteem vaak zonder schade doorstaat. Sommige grotere (menselijke) storingen kunnen echter niet getrotseerd worden, met als gevolg dat het ecosysteem ontwricht wordt. Het is daarom waardevol om te weten in welke staat een ecosysteem verkeert en wat het gevolg van potentiele verstoringen kan zijn. Een algemeen geaccepteerde methode om de staat van een ecosysteem te beschrijven is met behulp van een voedselweb(structuur). Een voedselweb is een diagram dat de wie-eet-wie relaties in een ecosysteem laat zien. Voedselwebstructuren kunnen ons, geanalyseerd over tijd en ruimte, informatie geven over de effecten van verstoringen en biodiversiteitsverlies, de gevoeligheid van soorten voor ingrepen en verstoringen en de mate van verbondenheid tussen soorten (connectivity).

Reconstructie van voedselwebben, met behulp van stabiele isotoopanalyses, kunnen gebruikt worden om de staat van een ecosysteem te bestuderen. Analyse van stabiele stikstof en koolstofisotopen is een krachtige en veelvoudig gebruikte methode om voedselwebben te reconstrueren. Stabiele isotopen worden gebruikt om de voedselwebpositie van een soort of soortsgroep te bepalen, om vervolgens een tweedimensionaal voedselweb op te stellen. Het stabiele stikstofisotoop ($\delta^{15}N$) wordt gebruikt om het trofische niveau te ontrafelen en wordt weergegeven als de verticale as van de voedselweb grafiek. Het stabiele koolstofisotoop ($\delta^{13}C$) worden juist gebruikt om aan te geven in welke mate een consument gebruik maakt van de verschillende primaire voedselbronnen aan de basis van het voedselweb en wordt weergegeven als de horizontale as van de voedselweb grafiek.

Zo op het eerste gezicht lijkt het Waddenzee ecosysteem wellicht een tamelijk ongestoord soortenrijk systeem, maar dit is – althans in vergelijking met vroeger – verre van waar. De afgelopen 1000 jaar verloor het Waddenzee ecosysteem een deel van haar voedselwebcomplexiteit, veelal veroorzaakt door menselijke invloeden. Bescherming en herstel zijn tegenwoordig gelukkig een belangrijke prioriteit voor het beheer van het Waddengebied. De belangrijkste processen die leiden tot en gelijktijdig de voortgang beschrijven van een stabiel en gezond Waddenzee ecosysteem zijn waarschijnlijk; (1) herstel van de sterk gedaalde en eens zo belangrijke en talrijk-voorkomende biobouwende zeegrasvelden, mosselbanken en zelfs oesterbanken die aan de basis van het voedselweb stonden en (2) de terugkeer van top-predatoren. Het is waardevol om meer inzicht te krijgen in de huidige staat en het herstel van het Waddenzee ecosysteem. De studie naar (verandering in) de vorm van de voedselwebstructuur van de Waddenzee kan ons helpen voorspellen hoe het systeem zal reageren op toename of afname van soorten(aantallen) of dieetveranderingen. Voedselwebreconstructie van het Waddenzee ecosysteem is nuttig bij het volgen van de veranderingen (van verstoring of herstel) in de Waddenzee en is cruciaal voor tijdig ingrijpen.

Dit proefschrift gaat over de complexiteit van voedselwebben, hetgeen ik met name door middel van stabiele isotopen onderzocht. Wie eet wie in een ecosysteem, en is dit te vertalen naar een – over tijd en ruimte geïntegreerd – voedselweb? Hoe kan voedselwebonderzoek waardevol zijn? Ik zocht naar de mogelijkheden om de voedselwebposities van indicatorsoorten te gebruiken als graadmeter voor de (ruimtelijk) variabele staat van het Waddenzee voedselweb.

Opbouw van het voedselweb

In **Hoofdstuk 2** laten we zien hoe een voedselweb ontstaat en verandert door de tijd. Dit bestuderen we door middel van de reconstructie van het voedselweb van de kwelder van het Waddeneiland Schiermonnikoog, met een focus op de rol van externe nutrienten. We maken gebruik van een honderd jaar oude kweldersuccessie, waardoor we het voedselweb van nul tot honderd jaar oude kwelder kunnen bestuderen. We vonden dat de jonge kwelder nog erg afhankelijk is van de toevoer van externe nutrienten uit het naastgelegen ecosysteem, de Waddenzee. Detritivoren (organismen die het afval van of dode organismen eten) worden hier gevoed door een externe nutrientenbron, de zee, en de detritivoren worden gegeten door carnivoren. Pas later in de successie wordt het voedselweb ‘klassiek’, met een interne nutrientencyclus, waarbij planten worden gegeten door herbivoren, die weer gegeten worden door carnivoren en waarbij de detritivoren al het dode materiaal verteren en zo zorgen voor een gesloten voedselcyclus. De opbouw van een voedselweb vereist dus interactie van nutrienten en energiestromen tussen ecosystemen.

Om het mariene Waddenzee ecosysteem te begrijpen is het waardevol om te weten hoe autonoom dit ecosysteem is en door welke nutrientenbronnen het gevoed wordt, zoals externe bronnen of benthische of pelagische algen (resp. van buiten het Waddenzee ecosysteem, op de bodem levende mariene algen en in het zeewater zwevende algen). In **Hoofdstuk 3** laten we zien dat het het Waddenzee ecosystem hoofdzakelijk intern gevoed wordt door locale benthische algen en dat het bovenliggende voedselweb hoofdzakelijk van deze benthische energie afhankelijk is. Daarnaast vonden we een hoge ruimtelijke variatie in de koolstofisotoopwaarden van benthische algen. Dit laat zien dat ruimtelijk gevarieerd monsters verzamelen van de benthische en pelagische algen belangrijk is om een eerlijk beeld te krijgen van (de basis van) het voedselweb. Gedetaileerde representaties van het Waddenzee voedselweb zijn hierdoor lastig weer te geven.

Beperkingen van voedselweb en isotoopanalyses

We ontdekten dat er, naast het ruimtelijk heterogene fundement van het voedselweb, meer complicerende factoren zijn die het representatief opstellen van een voedselweb bemoeilijken. Het gaat hier om veranderingen door tijd, ontogenie, leeftijd en mobiliteit van soorten, soorten die afhankelijk zijn van meerdere ecosystemen en de complexiteit van meetmethoden. In Hoofdstuk 4, 6 en 7 onderzochten we hoe zo eerlijk mogelijk een voedselweb(positie) bepaald kan worden.

In **Hoofdstuk 4** laten we zien dat de basis van een voedselweb ook voor stikstofisotopen ruimtelijk heterogeen is en dat de horizontale positie van soorten in een voedselweb afhankelijk is van het correct bepalen van de (gemiddelde) basis van het voedselweb. Mobiliteit en de onderliggende benthische of pelagische baselines (basis) van een soort kunnen daarom bepalend zijn bij correct schatting van trofische posities. Of er bij de bepaling een trofische positie rekening dient te worden gehouden met de ruimtelijke variatie aan de basis van het voedselweb (baseline), verschilt per soort en hun mobiliteit en onderliggende benthische of pelagische baseline.

Ook de levensfase waarin een individu verkeert (ontogenie) kan zeer bepalend zijn voor de voedselwebposities. Ter illustratie zien we in **Hoofdstuk 7** de toename in isotope stikstofwaarden naarmate de **kokkel** groter wordt. Kennis over welke grootte kokkels een kokkel-consumerend organisme eet is dus van invloed op de bepaling van de trofische postie. Verder laat het zien dat een soort in het voedselweb een variërende positie kan hebben in het voedselweb, al naar gelang zijn ontogenetische fase.

Ook de een correcte meetmethode kan cruciaal zijn bij de bepaling van een voedselwebpostie. (1) Individuen binnen één soort kunnen verschillende diëten hebben. (2) Verschillende weefsels van één individu geven een dieetbeschrijving variërend van de laatste dagen tot de laatste maanden of jaren en daarmee verschillen de isotoopwaarden per weefsel. (3) Het meetproces in het laboratorium is ook van invloed. Meet je het gehele monster, enkele lipiden of slechts een paar eiwitten? In **Hoofdstuk 7** laat ik aan de hand van harders zien hoe de trofische positie afhankelijk is van de meetmethode. Welke meetmethoden geschikt zijn, is afhankelijk van de onderzoeksvraag.

In **Hoofdstuk 6** laten we zien dat sommige soorten slechts periodiek gebruik maken van het Waddenzee ecosysteem. Drieteentjes wisselen van dieet tijdens hun seizoenstrek. Als zij aankomen in de Waddenzee bevatten zij nog weefsel met een oorsprong van een eerdere locatie waar zij foerageerden langs hun migratieroute. Dit onderzoek laat zien dat het voedselweb van tijdelijke (migrerende) soorten in een ecosysteem een speciale positie innemen in het voedselweb. Ze voeren materie aan van elders (poep), hun gemeten isotoopwaarden en dus dieet kan nog van een eerdere locatie zijn en ze verblijven slechts tijdelijk in het ecosysteem. We laten gelijktijdig ook zien hoe krachtig isotoopanalyses kunnen zijn, want door middel van één isotoopmeting (van twee weefsels) van een individu bemonsterd op locatie C kan zijn migratieschema achterhaald worden van locatie A naar B naar C.

Een voedselweb is statisch, maar de soorten in dit voedselweb zijn niet statisch, zelfs niet onder onverstoorde omstandigheden. We zouden dus eigenlijk graag een voedselweb over tijd en ruimte willen beschrijven. Tot op zekere hoogte kunnen we rekening houden met variatie over tijd en ruimte, want doordat hogere trofische organismen vaak een hogere levensverwachting en hogere mobiliteit hebben, zullen soorten hoger in het voedselweb deels de verschillen op kleinere schaal uitmiddelen. Voor soorten die zich niet kunnen voortbewegen en veelal onderin het voedselweb voorkomen, gaat dit niet op en kunnen grote verschillen bestaan in het voedselweb door tijd en ruimte. Om de genuanceerde processen die spelen bij verstoring of herstel goed te kunnen analyseren is echter een gedetailleerd plaatje van het ecosysteem vereist, een algemene beschrijving van een voedselweb zal hier onvolledig zijn. Mijn **conclusie** in de algemene discussie is dan ook dat voedselrelaties in een ecosysteem complexer kunnen zijn dan weer te geven is in een enkel voedselweb.

Kracht van isotoopanalyses bij voedselweb studie

Toch kunnen we leren van studies naar het voedselweb met behulp van isotopen. Ten eerste, kan het gebruik van indicatorsoorten veelbetekend zijn voor specifieke voedselrelaties binnen een ecosysteem. Isotoopmetingen kunnen inzicht geven in onbegrepen processen binnen een deel van het voedselweb. In **Hoofdstuk 5** laten we zien hoe de lepelaar als indicatorsoort kan dienen voor de staat van de kleine (plat)visjes die leven in de ondiepere delen van het Wad. De laatste decennia kwamen lepelaars terug naar de Waddeneilanden om te broeden. De omstandigheden op deze eilanden lijken vrijwel ideaal; met voldoende broedplaats, zelfs een toenemend aantal broedkolonies, weinig predatie en verstoring en schijnbaar goede voedselomstandigheden met veel foerageerruimte en zelfs een toename aan garnalen (de vermeende belangrijkste prooisoort). We onderzochten de veel herhaalde aanname dat garnalen het hoofdvoedsel zijn van lepelaars in hun broedperiode in de Waddenzee, door het dieet van lepelaarkuikens te onderzoeken door middel van kotsanalyses en isotooponderzoek. Beide methodes toonden aan dat niet garnalen, maar kleine (plat)visjes de belangrijkste prooi zijn tijdens het broedseizoen. Het lijkt slecht te gaan met de kleine platvisjes, wat hoogstwaarschijnlijk de oorzaak is van de afvlakkende lepelaarpopulatie en de dalende kuikenoverleving op de Wadden. We voorspellen dat herstel van jonge (plat)vis dichtheden in de Waddenzee terug te vinden zijn in het dieet van de lepelaarkuikens. Hiermee kunnen lepelaars dus als indicatorsoort dienen voor de stand van de kleine (plat)visjes stand in de ondiepe delen van de Waddenzee.

In **Hoofdstuk 7** laten we de kracht van isotoopanalyses bij voedselwebstudies ook zien door een dieetstudie van rosse grutto's en kanoeten. Isotooponderzoek blijkt een elegante methode om te bepalen welke wormsoort rosse grutto's prefereren. De prooidierkwaliteit van kanoeten blijkt in een $\delta^{13}C \sim \delta^{15}N$ bi-plot inzichtelijk te worden gemaakt door een glijdende schaal van links naar rechts. Zo zagen we dat ervaren kanoeten een dieet met kwalitatief hogere prooidieren hebben. Het lijkt er dus op dat onderzoekers en beheerders inzicht kunnen krijgen in de beschikbare prooidierenschelpen voor kanoeten (kokkel, wadslak en nonnetje) door de koolstof- en stikstofisotopen van kanoetenbloed te bepalen. Kanoeten zouden zo als indicatorsoort kunnen dienen voor de beschikbaarheid van schelpdieren met een hoge kwalitatief.

Ten tweede, kan een algemeen voedselweb gebruikt worden om ecosystemen te vergelijken. In **Hoofdstuk 7** vergeleken we vijf intergetijde ecosystemen in Europa en West-Afrika langs de Oost-Atlantische migratieroute. De intergetijdegebieden Waddenzee en Banc d'Arguin lijken anders gereguleerd met minder invloed van landbouw en met een minder marien signaal. Het intergetijdegebied van het Taag estuarium lijkt verrijkt te worden door externe inlandse stikstofbronnen. Hoewel er tussen de vijf intergetijdegebieden dus verschillen zijn, zijn er ook zeker overeenkomsten zoals bijvoorbeeld een brede basis aan primaire bronnen (brede $\delta^{13}C$ basis).

Conclusie

Resumerend, blijkt het zeer moeilijk of onmogelijk om een over tijd en ruimte geintegreerde weergave van wie eet wie in een ecosysteem (een compleet voedselweb) te geven. Dit omdat er teveel complicerende factoren een belangrijke rol spelen om alle voedselrelaties in één voedselweb te omschrijven, zoals; verandering door tijd, mobiliteit van soorten, verschillen binnen soort (zoals dieetwissels door verandering van leeftijdsfase en grootte), soorten die afhankelijk zijn van meerdere ecosystemen en complexiteit van meetmethoden. Een voedselweb kan wel waardevol zijn om algemeenheden te bestuderen, bijvoorbeeld om verschillen tussen ecosystemen te duiden. Voorts kan het bestuderen van individuele voedselwebposities (van indicatorsoorten) als graadmeter dienen voor de variabele staat van het Waddenzee voedselweb.

Ik eindig mijn proefschrift met een ietwat filosofische en hopelijk ongegrond pessimistische kijk op wat er zal gebeuren met het kleine stukje van de Zuiderzee wat nog over is, de Waddenzee. Ik koester dit stukje Nederland en bewonder haar veerkracht, maar vrees de nonchalance waarmee de mens de natuur beinvloedt.

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Mijn tijd als promovendus is mij dierbaar geweest en gelijktijdig ook best zwaar. Toen ik besloot dat ik deze PdD dolgraag wilde, leek het mij een goed idee om het totale voedselweb met behulp van stabiele isotopen te willen ontrafelen. Tot op de bodem. Ik ging als een razende van start, heerlijk. Maar het bleek wel erg veel, vooral aangezien ik het niet kon laten om alles in detail te bekijken, ik niet graag compromissen binnen mijn eigen werk maak en de Waddenzee een verdraaid complex systeem is. Gelukkig kreeg ik veel steun, zowel van mijn 'wetenschappelijke' als mijn echte familie.

Myn wittenskippelike heit dat bisto eins, **Theunis**, en dat hast yn myn eachen mei gloede dien. Ek al is dyn libben fansels oergriselik drok, wiedest dochs belutsen en geduldich, sawol op wurkflak as persoanlik. Koedest my (relatyf) makkelik wer op it goeie spoar sette – alhoewol 't ik flink koppich bin – en likest myn wrakselingen te begrypen of yn els gefal stjoere te kinnen. Hah, do seachst sels kâns om myn oerdriuwne each foar de lytste details of just de te grutte ideeën te temperjen. Dan seidest do dat ik myn ideeën mar 'efkes keurich opromje moast yn in doaske'. Fierder makkest my – noch hieltiid – razend entûsjast. Nei oerlis mei dy koe ik hast net wachtsje om wer fierder te wurkjen. Sels doet ik my sa rot fielde dat myn dosintewurk net mear slagge en ik my needsaakt fielde om te stoppen mei myn PhD, doe wistesto my noch oer te heljen om fierder te gean.

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Han, ik heb ontzag voor je brede en convergerende manier van denken, je luistert even een tijdje en lijkt het 'systeem' vervolgens volledig te kunnen doorgronden. Ik was dan ook erg blij dat je halverwege mijn tweede promotor werd. Het was een feest om overleg met je te hebben (hoewel dit jammerlijk weinig voor kwam, want je was vaak druk bezet of ik was niet in Groningen). Dan leerde ik zoveel en vertrok ik vaak met nieuwe ideeën voor mijn proefschrift.

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Gedurende mijn promoveertijd zijn er maar liefst vier post-docs binnen project Waddensleutels werkzaam geweest. **Tjisse**, do wiedest post-doc I en we sjouwden fan eilân naar eilân mei krabbenfallen en guod om diatomee mei te ekstraheare. Tuskentiids sietest do faaks op dyn mac kleptop, lies ik artikelen of makke in nei skepnet. Do moastest fansels it kokosmatte-eksperimint dwaan, ik holp wat, en letter moasten we dit yn de gaten hâlde. Drok, trochtocht en hastich gongen we te wurk. Dankewol, do joechst my in moaie start. Al gauw kreeg Tjisse een plek in Nijmegen en zo kwam post-doc II. **Marjolijn** regelde het mosselbankenexperiment, dit leverde een bak aan pracht data op, merci. 't Was mooi en hard werken, hè Marjolijn? Ik bewonder je flexibiliteit en vindingrijkheid. Ik was inmiddels al zwanger van Wout en had ook veel eigen onderzoek, waardoor we eigenlijk niet eens veel (veld)werk samen hebben gedaan. Dankjewel dat je tussendoor tijd voor mij vrij maakte zodat we mijn 'eigen' werk konden bespreken. Iets later werd jij ook zwanger en tijdens jouw verlof kwam **Els** even als vervanger, ergo post-doc III. Dank, Els en Marjolijn, dat jullie probeerden om mijn proefschrift op poten te zetten ('welke hoofdstukken wil je per se?'). Ik verzoop namelijk in alle opties en projecten. Aan **Laura**, mijn post-doc IV, ben ik ook veel dank verschuldigd. Je ben zo voorzichtig en vriendelijk in omgang en zo ambitieus en gedreven in je werk, dat ik flink tegen je opkijk. Dankjewel voor de vele tijd die je met me aan het baseline-stuk hebt besteed. Wat een klein makkelijk en ondersteunend stukje over stikstof-baselines moest worden, mondde uit in een analyse die ons volgens mij meer dan drie jaar achtervolgde. Het klein baseline-stukje bleek totaal niet zo eenvoudig en rechtlijnig als we hadden gehoopt, toch valt in hoofdstuk vier volgens mij een goed manuscript te lezen. Heel waardevol ook dat je meelas in de fase van het afronden van mijn proefschrift (algemene introductie en discussie), mijn dank dank dank.

Als er iets is wat ik veel heb gedaan tijdens mijn promotietijd, dan is het wel isotopenmonsters verzamelen, alleen maar vooral ook met collega's van het NIOZ. Hele kisten met kleine potjes en epjes hebben we verzameld (want heel het Waddenzee voedselweb, op vele locatie, over meerdere jaren). Ik heb erg goede – en ook ietwat spannende – herinneringen aan de mistnet experdities op het Wad (Richel, Hengst, Griend en met de Wadtoren, de Nav of vanaf Griend). Dolgraag zou ik nog eens mistnetten (als mijn kindjes iets groter zijn misschien). **Bernard**, dankjewel voor alle kennis die je mij gaf over vogels vangen en ringen aflezen. Ik vond het prettig om met je samen te werken en miste je dan ook echt toen je bij het NIOZ wegging. **Anne**, do ûndersochst faaks de mientse fûgelmagen mei in echo, mar ik herinnerje my noch datsto my op in lange nacht mei in grutte fangst ek noch kaamst helpen mei bloedprikken, super. **Sander**, naast het vele SIBES werk waar jij, Maarten, Anne en Job de draaiende motor van zijn, verzamelde je voor Waddensleutels (dus ook voor mij) extra monsters. Mijn dank, onderandere ook voor het verzamelen van extra diatomeeën en mossels en pokken van de vaargeulboeien (met je prachtige zelfontworpen schraper). Veldwerk op zee dat moet met een boot. Ik ben de **bemanning** van de *Navicula* (Bram, Hein, Tony en Wim-Jan) en schipper Ewout van de Stern dan ook dankbaar voor het vervoer. Het is vast niet altijd makkelijk om ons – vaak bevlogen en jachtige onderzoekers – aan boord te accomoderen.Ook mijn dank aan mannen van de Harder en Stormvogel, zoals Theo en Nico, van het EL&I.

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