Chapter 8

General discussion

Interest of marine ecologists in the direct interaction between harpacticoids and bacteria originated from the following facts: (1) as a basal food source, benthic bacterial biomass is (equally) high compared to MPB biomass (van Oevelen et al. 2006b) while its general fate remains unclear, (2) in view of their high densities, meiofauna may play a significant role in the transfer of bacterial biomass to higher trophic levels and (3) the current lack of evidence of strong predation of harpacticoids upon protozoa relative to the more numerous observations of the high attractiveness of bacteria-rich substrates for harpacticoids (e.g. Hicks 1977, Ravenel & Thistle 1981, Dahms et al. 2007), might point towards a direct pathway of bacterial biomass transfer to harpacticoids.
Exposing linkages between the microbial and classic food web will significantly improve our understanding of energy flow in marine benthic food webs and the overall functioning of coastal ecosystems, which is desirable in the current era of rapid environmental change.

This PhD study aimed to explore the two-directional interaction between harpacticoid copepods and bacteria. The thesis dealt with three main topics, which will be discussed as such.

In the first part of the thesis, we focused on the overall trophic diversity of intertidal harpacticoids and revealed the presence of bacterivorous species belonging different harpacticoid assemblages (chapters 2 and 3). In this general discussion, the major findings are discussed from an ecological point of view, denoting the implications of the observed harpacticoid trophic diversity for the connectivity between benthic food webs, i.e. the classic, the microbial and the detrital food web with emphasis on the consequences for the transfer of bacterial biomass. Secondly, under laboratory conditions the mechanisms of bacterial uptake (substrate dependence, feeding selectivity) was analysed in combination with the nutritional role of bacteria for harpacticoid copepods (chapters 4 and 5). Bacterial feeding and impact on copepods' health status was overall inferred from their natural stable isotope signature and fatty acid profiles and from the change in these trophic markers resulting from laboratory feeding. This part of the discussion is more fundamental and from the perspective of the harpacticoid grazer, addressing harpacticoids' (species-specific) feeding behavior towards bacterial biomass and the nutritional role of bacteria and other consequences of bacterial feeding for harpacticoids. Additionally, some methodological considerations about the applicability of trophic biomarkers for studying bacterivory were raised.
In third, the thesis examined the mechanism of early copepod fecal pellet degradation by bacteria and introduced a new method for visualization of fp degradation. More specifically, the importance of internal fecal pellet bacteria, which originate from the copepod gut as undigested food-related bacteria or resident gut bacteria, were of significant importance to fp degradation (chapter 6 and 7). Exactly these fecal pellets form a link between the classic and detrital food web but as copepods excrete active bacteria with their fecal pellets, the focus of this part of the discussion involves the feedback between the classic and microbial loop.

In the end, some general conclusion are presented and some future perspectives are considered.

HARPACTICOID TROPHIC DIVERSITY IN AN ESTUARINE INTERTIDAL AREA AND THE LINK BETWEEN THE CLASSIC AND MICROBIAL FOOD WEB

To explore the trophic diversity of meiofauna, estuarine intertidal areas are excellent study areas as they cover a wide spectrum of resources, habitat types and meiofaunal communities. Based on the trophic

marker analyses (chapter 3), we concluded that microphytobenthos (MPB) grazing was undeniably the predominant feeding strategy for harpacticods. This is in different microhabitats, with characteristic abiotic variables, various food availability and carbon inputs and comprising distinct harpacticoid communities and difference food condition (e.g. food availability and food quality in tidal vs salt marsh).
A MPB-based food web is a general characteristic of intertidal benthic food webs (Chanton & Lewis 2002, Pinckney et al. 2003, van Oevelen et al. 2006b). The high-nutritional MPB, with a dominance of diatoms, was widely distributed in the Paulina intertidal area but salt marsh stations are at the same time also dominated by retention of deposited low-quality suspended organic matter (SPOM), vascular plant detrital matter and high bacterial densities. Despite these depositions, harpactoids did not show an increase in reliance on detritus at a certain moment. This is in agreement with Van Oevelen et al. (2006) stating that meiofauna and macrofauna, are highly selective for high-qualitative MPB and hence, detritivory in an interitidal system is nearly absent. As a consequence, this means that total organic matter is not an appropriate measurement of foodavailability.

Food availability in the sand flat was lower compared to salt marsh stations (cf. low OM, phytopigment and protein+lipid concentrations). We suggest that the species-poor and low-density harpacticoid assemblages from tidal flats are primarily structured by granulometry and tidal exposure (abiotic factors), while assemblages from salt marshes are more bottom-up controlled by food quality and quantity. The primary impact of granulometry on the copepod assemblage is highly visible in station H1. There, the change in granulometry resulted in a drastic change in copepod community. H1 copepod species (*Tachidius discipes*, *Amphiascus sp. 1*, *Asellopsis intermedia*) correlated to nearly the whole range of habitat factors, indicating the overall change of the habitat and the copepod community due to a change in granulometry. Only *Ectinosoma* sp. (Ectinosomatida) showed only a single correlation. This fits well with the idea of Ectinosomatidae being independent of MPB. Moreover, fatty acid profiles of this family were very poor in diatom-related fatty acids (EPA, 16:1ω7). For this harpacticoid taxon, dietary information was conform the variation in its distribution pattern. Exept for Ectinosomatidae, harpacticoid species distribution patterns and correlation with resource variables, revealed relatively little about the trophic structure of the harpacticoid community and the governing factors that regulate spatio-temporal variation. In contrast to nematodes, there is little interest for microscopic examination of species mouth parts due to the weak link between mouth part morphology and food source consumption (Marcotte 1977, De Troch et al. 2006). Nevertheless, mouth-morphology based feeding type classifications of nematodes in tidal flat areas are also often little informative on the main drivers of assemblage structure and on the main resources used. Spatial isotope studies on nematodes from the Paulina intertidal area have, however, confirmed that nematodes also predominantly rely on MPB-derived carbon, and this across habitats (Moens et al. 2002, Moens et al. 2005a, Bezerra & Moens unpubl.). However, the results gradually shift from a 'pure' MPB signature in sandy and hydrodynamically impacted stations like H2, to a more mixed isotopic composition which reflects an increased contribution of SPOM-derived carbon in accretory stations such as muddy bare tidal flat stations and, particularly, salt marsh gully stations (Moens et al. 2002). Nevertheless, $\delta^{15}N$ data demonstrate that a considerable proportion of nematodes obtain part or most of their MPB-derived carbon indirectly, i.e. through feeding on an intermediate trophic level which in turn utilizes MPB (Moens et al. 2005a, Moens et al. 2013). Other lines of evidence for a strong bottom-up effect of MPB on tidal nematode assemblages at the Paulina come from (a) a field experiment on the recolonization of defaunated sediment (Van Colen et al. 2009), and (b) from the spatial correlations of nematodes with phytopigment concentrations (or ratios of pigment concentrations) (Moens et al. 1999a). In the former study, strong MPB blooms on previously defaunated muddy tidal flat sediments triggered sudden population peaks of presumed diatom-feeding nematodes like *Ptycholaimellus, Chromadora* and *Daptonema* (Van Colen et al. 2009). To what extent the absence of 'normal' populations of bioturbating macrofauna, which may interfere with diatom grazing by nematodes (Braeckman et al. 2011), or even prey upon nematodes (Olafsson 2003), contributed to this increase, remains unknown. In terms of their nematode assemblages, sandier baretidal flat stations at Paulina are characterized by high abundances of (large) predacious nematodes (Gallucci et al. 2005), the activity of which has the potential to exert significant top-down controls over prey nematode (and perhaps also ciliate, see Hamels et al.

2001) assemblage abundance and composition (Moens et al. 2000, Gallucci et al. 2005). The importance of top-down controls, both from hydrodynamic impacts and from predation, is therefore likely much more pronounced in bare sandy stations than in salt-marsh associated stations. Whether harpacticoid copepods in bare sandy sediments are also more prone to suffer from predation is unclear. However, given the high nutritional quality of copepods for higher trophic levels (Gee 1989, Heath & Moore 1997) and the nursery function of salt marshes to a variety of hyperbenthic predators of copepods (Cattrijsse & Hampel 2006), we can not simply assume that top-down impacts from predation on harpacticoid copepods would be less in marsh stations than on bare tidal flats.

In view of the concept of species co-occurrence and biodiversity maintenance (Arroyo et al. 2007), resource partitioning is expected to support the species-rich harpacticoid communities of the salt marsh stations. In fact, based on dietary markers (^{13}C and FA), we found that a few species (*Paronychocamptus nanus*, *Microarthridion littorale*, *Amphiascus sp. 1*) temporally relied on SPOM. For none of the species a considerable utilization of *Spartina alterniflora* detritus was denoted. Such a partial or temporary reliance on SPOM also occurs in nematodes from accretory station (see previous §). Utilization of *Spartina* detritus is difficult to demonstrate with natural carbon isotopes, since the *Spartina* signature tends to overlap with that of MPB. Studies which have suggested that *Spartina* may contribute to the diets of salt marsh nematodes and copepods (Couch 1989, Carman & Fry 2002) have indeed highlighted this problem. Nevertheless, the low nutritional quality of *Spartina* detritus, and the high similarity in carbon isotopic signatures of nematodes inside and away from *Spartina* vegetation, all suggest that MPB rather than *Spartina* detritus is the main carbon source for intertidal meiofauna in and nearby small salt marshes such as the Paulina marsh.

The above-mentioned partly indirect reliance on MPB carbon by a variety of tidal flat nematodes belonging to different trophic guilds (i.e., in essence, omnivory) (Moens et al. 2013, Bezerra & Moens unpubl.) suggests that many nematodes have some degree of flexibility in their diets, and may adapt feeding depending on the availability of different resources. Similarly, harpacticoids are generally considered being dietary flexible (Hicks and Coull, 1983). They can cope with a temporary depletion of MPB, for instance, in summer (despite high MPB productivity, Herman et al. 2001) resulting from extensive benthic grazing (Herman et al. 2000, Pinckney et al. 2003, Weerman et al. 2011). Even so, it is hard to explain why some of these species exhibit a dietary shift to the low-quality SPOM in the winter period since (1) still a considerable portion of MPB was available based on peaking of chlorophyll a in November and (2) SPOM deposition was typical in June. Copepod grazing rates adjust to changing MPB production (Pinckney et al., 2003) but that is not necessarily a reason for a drastic dietary shift. Moreover, in the salt marsh harpacticoid assemblages, bacterial consumption was documented for Cletodidae species and *Delavalia palustris* (family Miraciidae). For the other species of the copepod assemblage, small dissimilarities among species and small temporal variations in carbon isotopic signatures suggested selective feeding by copepod species or dietary shifts among similar food sources. Selective feeding by harpacticoids on different food sources was shown by Decho and Fleeger (1988) and more fine-scaled resource partitioning among different sources from the same food type was illustrated by e.g De Troch et al. (2006, 2012b) and Rieper (1982). However, *in situ* harpacticoid resource partitioning remains a rather unexplored topic.

Less expected was the trophic distinctness of species from the species-poor harpacticoid assemblage in the sand flat, comprising a diatom feeder (*Asellopsis intermedia*), a potential dinoflagellate feeder (*Tachidius discipes*) and also a bacterial feeder (*Paraleptastacus spinicauda*). In this case, resource partitioning does not function as biodiversity regulator but is presumably out of necessity due to relatively low food availability in physically stressed coarse sediments). Furthermore, field ^{13}C signatures showed that Harpacticidae, *P. spinicauda* and Cletodidae were confined to a single food source. Additionally, for the latter two taxa, their consistent ^{13}C-enriched and extremely depleted signature, respectively, combined with remarkable FA profile characteristics (bacterial FA, no diatom or MPB

markers) prove a high dependency on bacteria even when abundant diatoms are available (specialist feeders) and underlines their discriminate feeding on bacteria (fig. 1).

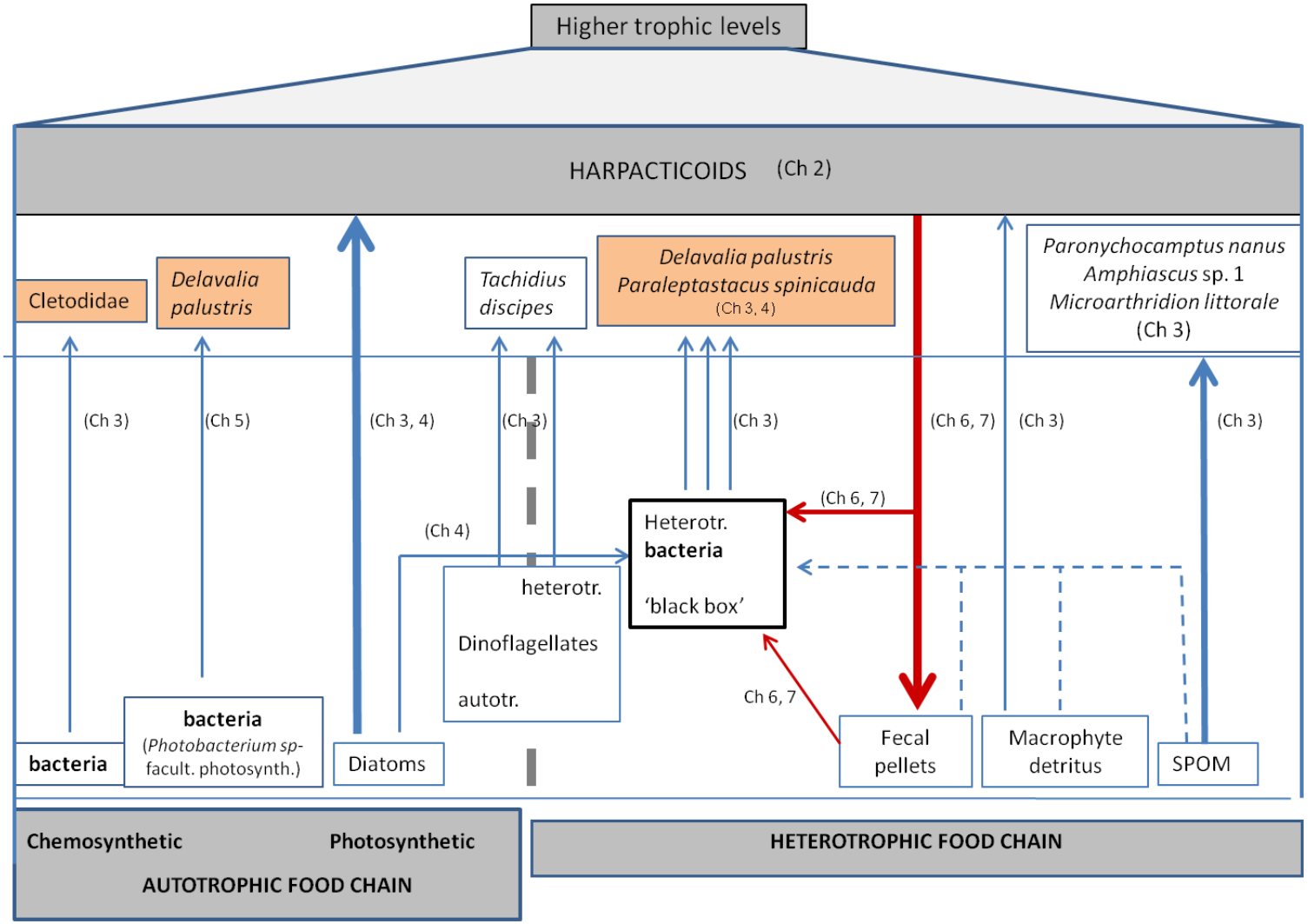

Fig. 1. Summary of major trophic linkages between harpacticoid and food sources that were covered in this PhD research. Specific chapters are indicated by numbers. Arrows represent observed interactions or potentially occuring interactions (dotted line). Line width illustrates the relative strength of the interaction (deduced from the number of literature reports, not quantified in this PhD thesis). Blue lines represent upwards fluxes of energy and red lines show the downward fluxes.

The combined use of dietary biomarkers, i.e. stable isotopes and fatty acids (FA), proved to be very useful to reveal differences in *in situ* food source consumption. However, we did encounter ambiguous results owing to the restrictions of stable isotopes and FA for *in situ* use (see further). Additional dietary biomarkers are needed to increase the resolution and to unravel food utilization, for example the use of FA biomarkers in the apolar fraction of lipids in consumers.

With respect to the ecological importance of bacterial feeding, our study covered a wide range of habitats and species and clearly showed limited assimilation of bacteria in terms of number of copepod species applying this feeding strategy. When compared to MPB, this implies a negligible contribution of bacterial production to the overall benthic trophic fluxes. However, the obtained results did not allow to make any strong suggestions on the relative importance of bacterial feeding opposed to some other food sources such as SPOM and protist feeding (dinoflagellates) (Walters & Moriarty 1993). The latter two were observed for only a few copepod species. Also the minute contribution of *Spartina* detritus to the diet of some species, suggests a poor energy flux of detrital matter (detritivory *sensu stricto*).

Van Oevelen et al. (2006a) and Epstein (1997) estimated that bacteria contribute 2-3% to a meiofaunal diet and bacterial carbon flux through meiobenthos grazing is merely 9% of total bacterial productivity. Van Oevelen et al. (2006a) found bacterial uptake by meiofauna to be high enough to be more than merely random uptake, but still too low to classify it as substantial bacterivory. It was concluded that transfer of bacterial carbon to grazers was limited (Kemp 1988, Epstein & Shiaris 1992) and that bacterial production still function as a carbon sink in food webs.

The few harpacticoid taxa with clear indication of trophic bacterial dependence were Cletodidae, *P. spinicauda* and *Delavalia palustris* (fig. 1). Highly depleted ^{13}C values typically points at the utilization of chemoautotrophic carbon (energy obtained from inorganic components), produced by bacteria. The lack of bacterial fatty acids, could imply that consumption of chemoautotrophic-derived bacterial-related carbon without actual assimilation of the bacterial cells, through assimilation of extracellular polysaccharide substances or bacterial derived DOM. Bacteria consumed by *P. spinicauda* are most likely epipsammic, autotrophic or heterotrophic bacteria while *D. palustris* consumed heterotrophic bacteria associated with diatoms. Despite the low harpacticoid diversity contributing to bacterial carbon transfer, the three copepod taxa showed to transfer bacteria between the chemoautotrophic, the heterotrophic and potentially also the photoautotrophic pathway in the estuarine food web (fig. 1).

HARPACTICOID BACTERIAL FEEDING: INGESTION, SELECTION AND ASSIMILATION OF BACTERIA

Harpacticoids are well-known substrate feeders and occasionally filter feeders. The high substrate-dependence for bacterial consumption reported in chapter 4, albeit only on a substrate with nutritional importance, therefore was expected. However, in addition to the consumption of bacterial biofilms associated with a high-nutritional substrate i.e. diatoms (mechanism 1) (Decho & Fleeger 1988, Souza-Santos et al. 1999), also two other mechanism have been recorded, namely grazing on bacteria associated with low-nutritional substrates (mechanism 2) e.g. copepod fp (De Troch et al., 2009, Arroyo et al., 2007), mucous substances (Hicks & Grahame 1979) and detritus (Perlmutter & Meyer 1991) and on bacteria associated with a non-nutritional substrate (coarse sediment grains) (mechanism 3)(Gray 1968). For the latter, the harvest of bacterial biofilms on sediment occurs via substrate scraping of coarse sediment grains or microbial stripping of ingested smaller grains inside the gut. In our experiment, the presence of silt grains as a pure physical substrate, however, did not enhance harpacticoid feeding on bacteria. However, we do not reject the *in situ* occurrence of this bacterial feeding strategy ('sediment grain grazing'). Particularly, a pure physical use of a bacterial-rich substrate is presumably applied by bacterial specialist feeders like Cletodidae from silty sediments and *P. spinicauda* from the sand flat. The latter most

likely applied grain scraping rather than grain ingestion considering the small copepod body volume:grain size ratio. Cletodidae may ingest silt grains followed by 'microbial stripping' in the gut. On the other hand, ingestion of a nutritional substrate with only the specific assimilation of the bacterial component is plausible and was once observed by Decho and Castenholz (1986), who noted copepod bacterial assimilation while co-ingested diatom cells were passed undigested to the fecal pellets. Finally, for *Delavalia palustris*, bacterial consumption is based on co-ingestion of bacteria and diatoms (mechanism 1), followed by co-assimilation, confirmed by both *in situ* (chapter 3) and experimental (chapter 4) fatty acid data showing considerable proportions of bacterial and diatom-specific FA. In contrast to *D. palustris*, other diatom feeders in the experiment (*Platychelipus littoralis*, *Microarthridion littorale*) did not show bacterial FA incorporation. Potentially, the signal of bacterial assimilation was too low compared to that of diatoms due to the large difference in biovolume. This would suggest that *D. palustris* obtained its bacterial signal by additional feeding on bacterial cells independently from diatom grazing. Based on the knowledge that copepod species, including *D. palustris*, are incapable of targeting bacteria in absence of a substrate, another explanation is needed. The difference in bacterial signal between *D. palustris* and other copepod species then points at a differential mechanism of food source assimilation. Copepods are able to actively enhance food digestion by means of regulating the gut transit time (see further, Tirelli & Mayzaud 2005). Moreover, little is known about the difference in digestibility of both food sources, diatoms and bacteria. Furthermore, we have no data at hand to exclude bacterial consumption through symbiosis. A third underlying mechanism of the trophic interaction with bacteria (in the case of *D. palustris*), can include the use of mucoid substances (mechanism 2). Similar to other mucus-producing harpacticoids inhabiting silty sediment (Hicks & Grahame 1979, Williams-Howze & Fleeger 1987) and e.g. mucus production by *D. palustris* (Nehring 1993)can be a strategy to 'garden' its own bacterial food. All this highlights the diversity in bacterial feeding strategies by intertidal copepods at the level of bacterial ingestion and of bacterial assimilation.
Finally, the inability of harpacticoid copepods to target 'loose' bacteria (chapter 4) is in line with the idea of harpacticoids' inability to capture and consume small-sized particles, independent of a substrate.

Considering the low assimilation efficiency of bacteria ingested by diatom grazers, only *Delavalia* showed to be a relatively efficient bacterivore. Although diatoms were assimilated by multiple intertidal harpacticoids (*Platychelipus littoralis*, *Microarthridion littorale*, *Nannopus palustris*), associated bacteria were not. Therefore, the fraction of random/co-ingested bacteria by most copepods does not contribute to the transfer of bacterial carbon through the food web.

Further, bacterial selectivity was proven by means of *in situ* observation of bacterial specialized feeders i.e. for Cletodidae and *P. paraleptastacus* (chapter 3). Similar to the selectivity of harpacticoids towards diatoms at the level of species, growth phase or cell size (De Troch et al. 2006, 2012b), it seems that harpacticoids discriminate among bacteria. Under experimental conditions, selective bacterial feeding was shown by Rieper (1982). However, this study is based on ingestion, while further assimilation was not estimated. In our experiment there was a slightly higher uptake of *Photobacterium* sp. by *D. palustris* (Chapter 5, Fig. 1). Bacteria offered were considered to be of different nutritional values (based on dissimilarity in fatty acid and protein composition). The experimental setup is rather restricted to draw general conclusions about harpacticoids' ability to select among bacteria and the possible causative factors for that e.g. nutritional content, depending copepod-species specific ability to process certain fatty acids.

The low and rather similar bacterial ^{13}C assimilation among copepod species pointed towards indiscriminate feeding on bacteria and random co-ingestion during diatom grazing (1996, Souza-Santos et al. 1999), but the higher bacterial fatty acid incorporation of *D. palustris* (bacterial bacterial constituting up to 23 % of total fatty acids) compared to the near lack of bacteria FA in other species (<5 %) (chapter 4) suggests copepod-specific differences in assimilation efficiency of bacteria. Harpacticoids may regulate gut transit time depending on the food quality to increase assimilation (Tirelli & Mayzaud 2005). The existence of differential digestion of resource among different copepod-species has not been reported for

meiofauna but was refuted for macrofaunal detritivores by Plante and Schriver (1998a, b): digestive fluid of multiple macrofaunal species showed equal bacterial cell lyses of 'ingested' sediment bacteria, but instead the food source characteristics determined their susceptibility to digestion (see also further).

NUTRITIONAL ROLE OF BACTERIA

Laboratory feeding experiments reveal assimilation of bacterial carbon and fatty acids but also confirmed the poor nutritional contribution of bacteria for harpacticoids relative to that of diatoms (chapter 4, 5). The majority of experimentally tested copepods, except for *Delavalia palustris*, clearly did not attain any nutrition from bacteria and copepods lived of their FA reserves in presence of bacteria as sole food source. However, feeding on a purely bacterial diet was not always found to result in a nutritional deficiency in the harpacticoid, since copepods were capable of normal reproduction (Rieper 1978). For *D. palustris*, the secondary importance of bacteria relative to diatom feeding, suggest bacteria to serve as a complementary food source, which is in line with the findings of Souza-Santos (1999) that supported the general idea that bacteria are co-assimilated with a high-qualitative food source without having a confounding influence on grazer nutrition, and may merely provide some dietary supplements such as vitamins.

However, a higher and constant dependence on bacteria and limited consumption of another food source found for Cletodidae and *Paraleptastacus spinicauda* (bacterial FA: up to 15 % of copepod FA content) suggest that the bacteria might be more than a complementary food sources. Moreover, in spite of the highly characteristic carbon isotopic signal of Cletodidae linking this species to a chemoautotrophic food source, the absence of bacterial FA in Cletodidae, might indicate the use of extracellular released compounds of chemoautotrophic bacteria (EPS, DOM), as mentioned before, instead of actual bacterial cell lysis in the gut and subsequent assimilation of bacterial cell biomass.

The poor nutritional value of bacteria is related to the lack of PUFAs and sterols, which are essential compounds for copepods (Ederington et al. 1995) as they contribute to somatic growth and cell membrane functioning and any shortage of these essential FA may limit zooplankton productivity. In addition to the FA composition of the food, copepods are selective towards protein-rich resources (Cowles et al. 1988) and other nutritionally important components such as amino acids, elemental composition (e.g. C:N:P ratios), vitamins and other trace elements (Dauwe et al. 1999, Touratier et al. 1999). Another remarkable finding is the differential biochemical response of *D. palustris* compared to the other species to poor-food-value conditions. In absence of a high-quality food source, all copepods including *D. palustris* lost FA within the first 4 days but the latter was able to recuperate during the following days of incubation and even biosynthesize essential FA (PUFA), through FA biosynthesis using bacterial-derived FA as precursors (chapter 3, 4). Clearly, in the absence of diatoms, also for *D. palustris* a limited nutritional contribution of bacteria is observed. As a potential stress response, a PUFA-production is thought to increase the survival rate of this species when it occurs in poor-food conditions.

DIETARY BIOMARKER ANALYSIS FOR THE STUDY OF BACTERIVORY: METHODOLOGICAL CONSIDERATIONS AND METHOD EVALUATION

Techniques used for quantifying copepod feeding are based on gut analysis, removal rates, fecal pellet production and pigment content of fecal pellets (e.g. Harris 1994, Azovsky et al. 2005, de Souza Santos & Castel 2013). However, neither of these are suitable for inferring bacterial grazing. Many studies promote the use of stable isotopes and FA for disclosing trophic interactions and carbon fluxes in the lower food web (Peterson & Fry 1987, Leduc et al. 2009). These dietary biomarkers are a relatively new and powerful

tool for direct measurements of assimilation. However, this approach has some drawbacks with respect to bacterivory (see below).

In the microcosm experiments, both types of dietary markers, stable carbon isotopes and fatty acids, provided direct information on bacterial uptake by harpacticoids. For revealing *in situ* food source consumption, copepod fatty acid signatures were highly suitable for inferring bacterial feeding while isotopic analysis informed on utilization of non-bacterial resources, demonstrating the added value of complementary use of fatty acids and stable isotopes.

In situ application of stable isotopes

Carbon isotopic signatures of bacterial biomass and their substrate (e.g. algae, detritus) overlap (Boschker & Middelburg 2002). For nitrogen, isotopic signatures are little predictable since bacterial ^{15}N fractionation depends on the molecular nature of the organic nitrogen source (see chapter 3) (Macko & Estep 1984, McCarthy et al. 2007).

Moreover, during simultaneous feeding on diatom and bacterial biomass (e.g. for *Delavalia palustris)*, diatom assimilation exceeded bacterial assimilation and copepod isotopic signatures will therefore resemble to those of herbivores, masking the bacterial uptake. Hence, the use of dual isotope analysis for discriminating between bacterial feeding copepods (e.g. *Paraleptastacus spinicauda* and *Delavalia palustris*) and copepods with another feeding strategy (e.g. herbivorous) was insufficient. In this respect, results from controlled experiments using ^{13}C-prelabelled bacterial food are less confounding.

In situ application of fatty acids

In situ copepod fatty acid (FA) profiles are characterized by a certain extent of natural variability, which obscures clear patterns of food utilization (detritus, MPB, SPOM). This results from spatio-temporal variability in resource FA, by copepod feeding on a mixture of resources and by copepod FA variability resulting from copepod species-specific behavior (breeding pattern) and FA metabolism (see chapter 3). This was more a concern for inferring feeding on non-bacterial resources based on indicator FA such as EPA and DHA. The highly specific odd-chained and branched bacterial fatty acids (15:0, 17:0, 15:1ω5, 17:1ω7) provided clear evidence of bacterivory in harpacticoid species. Additional use of the bacterial characteristic FA 18:1ω7, which is often proportionally more abundant than the odd-chained bacterial FA, would reinforce the bacterial signal of consumers markedly. In our study, this FA could not be identified due to restrictions in the detection resolution of the GC-column hampering the separation of 18:1ω7c and 18:1ω9c.

In this work, FA from total lipid extracts (polar and apolar lipids) were analysed. The use of the apolar fraction is a more ideal method for inferring dietary information since the other fraction i.e. the polar fraction (membrane FA) is highly dependent on genetic and environmental factors (e.g. temperature). The presence of FA derived from polar lipids thus reduces the value of the analyzed FA pool (from the total lipid extract) as trophic markers to reveal the nature of consumed food sources.

BACTERIAL FECAL PELLET DECOMPOSITION

Independent of their origin (field or lab conditions), **benthic fecal pellets are carriers of a diverse set of bacteria** that has survived gut passage, as visualized by AFM-LSCM. Internal bacteria maintained their activity, hence breaking down the fecal pellet content. The additional efforts made to include natural fecal

pellets from field captured copepods in this study proved to be of value, since they illustrate even better the diversity of active bacteria that is shunted to the fecal pellet and the natural variability in bacterial assemblage composition. Fecal pellets tested differed in food content, i.e. composed of unknown natural and likely mixed food sources versus a laboratory diatom food source composed of a single diatom species. Fecal pellets also originated from two different copepod species, i.e. a field copepod species and a second, laboratory-reared copepod species. Hereby, we excluded the possibility of only an artificial effect of fecal pellet enrichment with active bacteria.

Although a majority of bacterial phylotypes could not be unambiguously linked to the ingested food source or to copepod associated bacteria (i.e. in the gut or externally on the body), at least a fraction are undigested food bacteria. Furthermore, the easy and rapid addition of food bacteria to the gut flora of *Platychelipus littoralis* stresses the major influence of the type of grazed food source on both gut flora and bacteria fecal pellet content. These results underline the impact of copepod feeding behavior, including food source preferences and temporal dietary shifts, and the co-ingested bacterial fraction on the genetic diversity of active bacteria. Internal fecal pellet bacteria had a relatively wide metabolic potential, well suited to decompose a wide range of substrate types such as polymers, carbohydrates, etc. Corresponding to the genetic variability in fecal pellet bacteria induced by copepods' feeding behaviour, the metabolic potential of the bacterial assemblage might change, provoking differential fecal pellet degradation rates.

Moreover, benthic fecal pellets were less subjected to bacterial colonization from the surroundings, which could imply that recycling of benthic fecal pellets differs from that of planktonic fecal pellets. As a consequence of limited 'external' bacterial enrichment of the fecal pellet in terms of bacterial diversity, colonizing bacteria only limited contributed to the catabolic traits of the active bacterial community of the fecal pellet. This stresses the profound contribution of internal bacteria to fecal pellet degradation. This contrasts with the strong significant bacterial enrichment observed for planktonic fecal pellets (Jacobsen & Azam 1984, Jing et al. 2012). Reports on benthic fecal pellets are lacking but (natural) fecal pellets in sediment traps are in a way representative for benthic fecal pellets due to their static state. Also on these fecal pellets bacterial coating was poor (Gowing & Silver 1983). Consequently, degradation of benthic fecal pellets (or pellets from sediment traps) is not comparable to the degradation process of planktonic fecal pellets. Due to the static position of benthic fecal pellets and the concomitant limited exchange of oxygen, DOM or other chemical cues (produced by bacterial activity or leaked from the fecal pellet) (Ploug & Grossart 1999) compared to sinking fecal pellets, bacterial colonization is supressed. On the other hand, a more efficient bacterial exchange between the environment and the benthic fecal pellets would be expected as benthic fecal pellets are in close contact with other bacteria-rich matter such as sediment biofilms or detrital particles. Hence, undigested bacteria from the copepod gut and ingested food sources which are channeled to the fecal pellet, and not those from the seawater colonizing the fecal pellet, are the primary degraders of benthic fecal pellets. A considerable fraction of ingested bacteria has the potential to survive gut passage. For example, for macrodetritivores ingesting large amounts of sediment bacteria, 74% of sediment bacterial strains were resistant to digestion. The susceptibility of sedimentary bacteria to lysis by digestive fluid relates mainly to the cell wall type of bacteria (Plante & Shriver 1998a, b).

For a better comprehension of fecal pellet degradation, molecular tools for screening the bacterial diversity involved in the degradation process can be complemented with a visualization tool, for instance AFM-LSCM. AFM-LSCM allows more precise measurement of bacterial cell volumes (due to depth measurement) and the persistence of internal bacteria compared to the invasion by external bacteria can be closely followed and can visualize bacteria-bacteria interactions or the state of the peritrophic membrane. With the use of appropriate fluorescent markers, the changes in biochemical content can be mapped recording whether biomolecules (carbohydrate, chitine, lipids, etc.). Biochemical change of the copepod fecal pellet als fits in the topic of throphic upgrading.

GENERAL CONCLUSIONS

Trophic diversity of intertidal harpacticoids in the Paulina intertidal area was low in respect of the heterogeneity in habitats. The dominating role of microphytobenthos, in particular diatoms, as a food source for intertidal harpacticoid copepods compared to bacteria, suggests a negligible connectivity between the grazer food web and the microbial loop and denotes bacteria as a carbon sink in the intertidal ecosystem. Nevertheless, clear evidence of bacterivory for field harpacticoids has been found, giving us a wake-up call regarding their excistence. By revealing Cletodidae as a consumer of chemoautotrophic bacteria or bacterial-derived exudates, a new trophic link has been introduced for intertidal harpacticoids. From the few earlier observations of a chemoautotrophic pathway (see discussion chapter 3), copepod identification was still missing. In addition, consumption of bacteria by *Delavalia palustris* was continuously confirmed, from field and experimental data. Moreover, of four tested species, it was the only one that had the ability to biosynthesize PUFA during starvation. It remains unclear if this is some sort of adaptation related to feeding on bacteria but it can be expected that this strategy will not support *D. palustris* for a longer time period. Finally, contrasting to the previous two copepod taxa, *Paraleptastacus, spinicauda* is a bacterivore in the sand flat. These observations of bacterivory among harpacticoids suggest that (1) bacteria from different origin can be consumed (chemoautotroph, heterotrophy and potentially photoautotroph), that (2) bacterial utilisation is not confined to a single habitat type (muddy salt marsh and sand flat) and that (3) bacteria can be an additive to the diet of a herbivore or be the primary food source a a specialist feeder. Other dietary information from field copepods, such as the poor reliance on *Spartina* detritus, fits well with the study of Van Oevelen et al (2006b). Dietary information, was of no surplus value for explaining harpacticoid distribution patterns.

The bacterial assemblage of fresh copepod fecal pellets, located at the inside of the fecal pellet as confirmed by AFM-LSCM, denoted a reverse interaction between the grazer food web and microbial-detrital food web. A proportion of ingested bacteria, for most harpacticoid species co-incidentally ingested during grazing on a primary food source, returns to the microbial loop and this contributes to the concept of bacteria being a sink. Furthermore, the absence of intensive external colonization contrasts with observations from the planktonic system. It raises questions about the efficiency of degradation processes in the benthic ecosystem compared to the pelagic microbial-detrital food web

FUTURE RESEARCH

- Although the transfer of bacterial biomass seems at first limited and restricted to a few harpacticoid species, it is still interesting to continue this research and **exposing more bacterivorous (omnivorous) harpacticoid species** since the discovery of bacterivorous Cletodidae and Paraleptastacidae was also not expected. We need more exact data on the amount of bacterial biomass transfer and we currently might still underestimate this food web link. The bacterivore species from this thesis were fairly abundant, if not the dominant species from the harpacticoid assemblage (e.g. *Paraleptastacus spinicauda*, dominant in the sand flat community). Locally, depending habitat type, bacterial transfer can be higher than we would currently expect.

- No strong indication of **selective bacterial uptake** was observed, but more experimentation is needed, testing a broader range of nutritionally different bacterial strains.

- At the same time, I belief it is interesting to start investigating another way of bacterial biomass transfer, i.e. the interaction between **bacterivorous protists and harpacticoids**. This food web

link is nearly unexplored and it contributes to the still urging question of what the fate of bacterial production is. Protists are of higher food quality (high C content, through trophic upgrading) (Breteler et al. 1999) but can also be toxic to harpacticoids (Colin & Dam 2003). Omnivorous harpacticoids would benefit from protozoan grazing e.g. during periods of limited MPB.

- In addition, if indeed the microbial loop is a dead end in bacteria (van Oevelen et al. 2006b), we should start exploring the DOC-pathways, the direct link between **harpacticoids and DOC** (Cletodidae, feeding on DOC of symbiotic bacteria?) or through the microbenthos as intermediates. Van Oevelen et al (2006b) state that most protozoans from the intertidal feed on DOC rather than on bacteria.

- We introduced in this thesis (chapter 7) **AFM-LSCM** as a potential tool for visualizing **fp degradation**. It would be interesting to further exploit this technique, applying it to follow-up some fecal pellet characteristics during fp degradation: the state of the peritrophic membrane, the bacterial biomass on the inside and outside of the fecal pellet, the change in biochemical composition (carbonates, chlorophylls, chitine, …).

- I'm curious whether the limited colonization of seawater bacteria on benthic fecal pellets is a general phenomenon for benthic particles. This could have implications for the **degradation rates of benthic material**. To my knowledge, no studies have yet compared degradation rates of the planktonic and the benthic system.