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“You are what you eat”: How diet can influence the gut microbiota of marine invertebrates

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Microorganisms and their value to higher organisms

Microorganisms are omnipresent in every ecosystem on earth and the majority of identified species belong to the domains Bacteria and Archaea of which bacteria are considered to represent the greatest diversity (Staley *et al.*, 2007). Microbes are organised in complex and diverse communities depending on the environmental conditions, and they commonly live closely associated with eukaryotic hosts. The body surfaces of nearly all higher organisms are populated by diverse microbial communities and this microbiota is understood to have large impacts on the hosts' biology (Ley *et al.*, 2008). The greater part of the microbiota consists of microorganisms that are beneficial to the host and interactions between the host and its microbiota are often of symbiotic nature. For instance, the presence of bacteria can enhance the immune response of the host and protect it from other pathogenic microorganisms, and in return the host offers a habitat to the bacteria and provides them with nutrients (Fraune and Bosch, 2010).

Host-allied microbial cells outnumber the host cells considerably and the greatest percentage of the microbiota is made up by microbes colonising the stomach and digestive tract of the organism. Metagenomic analyses of the human gut-resided microbes revealed that bacteria dominate the gut microbiota and thus, the overall microbial genetic information -or microbiome- is mainly constituted of bacterial DNA (Gill *et al.*, 2006; O'Hara and Shanahan, 2006). In addition to the above mentioned benefits from the microbial symbionts to the host, many bacteria within the gut microbiota have important roles in the harvesting of energy from ingested food by producing enzymes that break down nutrients with more complex molecular structures. These exogenously synthesised enzymes are essential for the processing of numerous foodstuffs and in most cases the host is unable to generate them itself (endogenously). As an example, the genes coding for carbohydrate active enzymes (CAZymes), which are crucial elements for the digestion of polysaccharides and other molecules, were recognised absent in the human genome and were identified to come from present gut bacteria instead (Hehemann *et al.*, 2010). The microbiome, therefore, complements the genome of the host and supports important physiological activities and functions. Ray *et al.* (2012) reviewed the information gathered from several studies on the digestive enzymes produced by gut bacteria isolated from the gastrointestinal tracts of fish and likewise confirmed that enzymes produced by the bacteria were important for the digestion of certain dietary components such as plant-coupled cellulose. The bacteria analysed in the

reviewed studies showed cellulose activities and it was presumed that these gut bacteria support the digestion of plant materials in vertebrates other than humans.

By synthesising digestive enzymes, gut bacteria add greatly towards energy availability and uptake from food components (Tremaroli and Bäckhed, 2012), and this contribution was illustrated in gnotobiotic (germ-free) mice that displayed a lower energy yield compared to mice with an intestinal microbiota fed the same diet (Vrieze *et al.*, 2010). As a result, it can be concluded, that energy extraction and yield is directly linked to the activity, diversity and abundance of the microbial community inhabiting the host which subsequently influences its metabolism and can have effects on the fitness the organism.

Potential meaning of microbiota for the evolution of organisms

As mentioned, many microorganisms that live in symbiosis with a host play an important role in its physiology. Many higher organisms are even dependent on them in order to survive. Therefore, it is likely that the host-associated microbes may also have fundamental impacts on their evolution (Fraune and Bosch, 2010). Both host and microbiota are often considered as a single entity of selection, also known as the holobiont, of which total genetic information is referred to as the hologenome. The hologenome theory of evolution appreciates the microbial symbionts as important components of the host that can have an effect on the physiology, adaptation and Darwinian fitness and thus evolution of an organism (Zilber-Rosenberg and Rosenberg, 2008). This concept of evolution originated from a study of bleached corals that were suggested to have actively altered the composition of their symbiotic bacteria to more beneficial species with the aim of adapting to changes in the environment. Such selection for the most advantageous holobiont could explain the evolutionary success of corals over time where a change of environmental conditions occurred throughout Earth's history (Reshef *et al.*, 2006).

It is suggested that when studying the evolution of organisms that live in strong connection with microbial symbionts, the possibility of the hologenome should not be neglected as a factor influencing the host's biology. Sharon *et al.* (2011) provided evidence for the hologenome theory of evolution in studies of the fruit fly *Drosophila melanogaster* where an alteration in the animals' diet, and thus gut-associated bacteria evoked a change in the mating behaviour. Individuals treated with antibiotics failed to develop mating preferences, suggesting that the microbiota was responsible for this effect. The outcomes of this study underline how microbiota could influence the speciation and therefore evolution of organisms through the impact on mating preference.

Diet can form and alter the microbial gut community

Organisms are largely anticipated to be born germ-free (axenic) and microorganisms accumulating in the gut ecosystem of a host are attained from the surrounding environment, primarily via ingestion of food material and water. Studies investigating the origin of gut-inhabiting bacteria in *Drosophila melanogaster* revealed that freshly hatched and axenic fly larvae received their microbial symbionts from the eggshells they fed on, which were contaminated with bacteria from faeces of adult flies (Broderick and Lemaitre, 2012). The outcomes give reason to assume that the origin of indigenous gut bacteria lies in the intake of foodstuffs. In general, the gut microbiota can be distinguished between indigenous components (autochthonous), which are the permanent residents of the gut ecosystem, and the transient ones (or allochthonous), which are usually only temporarily present in the gut of an individual. Autochthonous

microbes colonise the gut mucus and many are engaged in the hosts' digestion or protection against pathogens. The allochthonous microbes in contrast are usually only present in the intestinal content and are excreted out of the system after some time (Ley *et al.*, 2006). Hence, strong connections between type of food consumed and the present gut microorganisms can be inferred, and many studies verified this further.

De Filippo *et al.* (2010) analysed and compared the gut microbiota of children from modern European and rural African human populations which differed in the type of diet due to cultural backgrounds. The diet of the African population was compared to the one of early human settlements with a high amount of fiber, starch and plant polysaccharides in the daily food consumption. Children from Europe on the other hand have been identified to eat a greater amount of animal proteins, sugar and fat, and generally much less fiber than children from traditional Africa. 16S rRNA gene analyses and comparisons of faecal samples from both groups disclosed significant differences in bacterial community structures within the guts of the individuals. For example, the samples from the African populations had a higher percentage of gram-negative over gram-positive bacteria, whereas the samples from European populations showed the opposite distribution. Overall, the gut microbiota of the African children was richer and more diverse, and De Filippo *et al.* (2010) suggested that diet is a dominant factor shaping the gut microbiota of individuals.

Similar studies involving aquatic animals were accomplished and provided evidence for a strong connection between diet and the gut microbiota of the organism. In natural aquatic ecosystems, seasonal fluctuations of environmental conditions such as currents can influence the availability of food resources to organisms. Strong currents cause mixing of nutrients in the water column which promotes the growth of lower trophic levels (e.g. phytoplankton) resulting in a high abundance of those organisms. The marine lobster *Nephrops norvegicus* feeds on other animals preferably; however, it can alter its dietary habits to suspension feeding if food resources are scarce. Meziti *et al.* (2010) deduced that seasonal variations in food supply could affect the gut flora of *N. norvegicus* and analysed the microbial communities of the intestines from lobsters collected at different months of the year. The authors extracted the 16S rRNA sequences and compared the bacterial diversity between the samples. Significant variations of gut-associated bacteria were found from samples of different seasons (and hence food quality and quantity), confirming diet type as an influencing factor of the gut-microbiota (Meziti *et al.*, 2010).

The marine gastropod mollusc *Haliotis diversicolor* is one of the key farmed abalone species in aquaculture and understanding its development and life style is of great commercial interest. A change in dietary preferences from larval stage to adulthood has been observed in *H. diversicolor* ranging from small food particles (e.g. diatoms) in the juvenile stage to larger food sources like red seaweed in adults. Zhao *et al.* (2012) put forward that by consuming food from different resources during its life cycle, a change in the microbial diversity in the gastrointestinal tract of the organism would be displayed accordingly. The authors identified the gut-bacterial communities of selected developmental stages using 16S rRNA gene sequences and revealed a significant difference of bacterial diversity between the developmental stages. For instance, bacteria of the genera *Bacillus* and *Pseudoalteromonas* appeared in highest abundance in the non-feeding larval stage, whereas the first-feeding stages were dominated by *Vibrio* sp. which are ubiquitous in the aquatic environment and potentially found their way to the gut through the ingestion of diatoms.

All of the above summarised studies emphasise the effect of diet type on the microbial diversity in the intestinal systems of organisms, regardless of the phylogeny. However, diet is not the only factor that can shape the gut microbiota in organisms. It has to be respected that other environmental factors such as temperature and, in particular in marine habitats, salinity can affect the occurrence of certain microbial groups in the first place. Moreover, the intestinal system of an organism is exclusive to few groups of microorganism due to extreme conditions like low pH, and the gut is considered as a selective environment (Sullam *et al.*, 2012).

Microbial biofilms as food resource for intertidal marine invertebrates

Marine invertebrates are generally small and the majority of species rely on food resources from low trophic levels (Kaiser *et al.*, 2011). For small pelagic invertebrates, the food source may be the zooplankton (carnivores), phytoplankton (herbivores) or both (omnivores). Intertidal and herbivorous invertebrates like gastropods inhabit rocky shores and are dependent on vegetation on the shore as a food resource. This includes microbial biofilms that coat the rocks on the shore which are of significant importance to intertidal grazers (Jenkins *et al.*, 2001).

Biofilms are biological systems and consist of aggregations of microbial cells among which bacteria are considered as key constituents. Depending on the surrounding environment and its conditions, such films can be made up of a great range of species. The development of biofilms is regarded as a common adaptation of many microbes, especially bacteria, at which the cell community benefits from interactions allowing them to flourish more successfully (Davey and O'toole, 2000). Complex biofilm assemblages, or microbial mats, commonly consist of several layers of bacterial communities. Interactions of the microorganisms have been examined and the recruitment of biofilms is ensued in virtue of quorum sensing, an important mechanism utilised by many microbes. Quorum sensing terms the communication between cells whereby signalling molecules are emitted and detected which evokes a density-dependent "decision" and subsequent behaviour of the organisms (Decho *et al.*, 2009). Such behaviour includes the settlement and formation of biofilms on surfaces, and when cells attach to surfaces, they produce extracellular polymers (or EPS) to form a matrix that functions as structural support, protection and enhances cell interactions (Decho, 2000).

Microbial biofilms can be found at a range of interfaces such as the solid-liquid interface on rocky shores where they are regularly identified as slimy layers covering rocks. Analyses of intertidal biofilms from temperate shores have revealed that a major percentage of the represented microbial cells are photo-autotrophic bacteria (mostly cyanobacteria). Narváez-Zapata *et al.* (2005) used molecular techniques to analyse the bacterial composition of biofilm samples from a rocky shore in the Southern Gulf of Mexico and discovered four major phyla; *Bacteroidetes*, *Actinobacteria* *Proteobacteria* and *Cyanobacteria*, of which the latter portrayed the highest abundance of species. Besides the bacterial cells, intertidal biofilms also contain microalgal organisms such as diatoms and macroalgal germlings, and it has been proven that these biofilms significantly contribute to primary production in coastal regions (Nagarkar and Williams, 1997).

For many intertidal grazers, epilithic biofilms on the shore are the main food resource and the nutrients available to the organisms are limited to what is found in the biofilms, which generally encompass high proportions of proteins, polysaccharides (e.g. from the

EPS matrix), lipids and other essential components of a healthy diet (Fernandes Da Silva *et al.*, 2008). In aquaculture in particular, where finding the most cost effective feeding method is a constant issue, the values of using biofilms as alternative feedstuffs have been recognised. In a study by Fernandes Da Silva *et al.* (2008), the nutritional quality of biofilms was evaluated and diatoms for instance, were confirmed as the major contributors to the protein content in the microbial community, reaching the maximal amount of proteins when the diatom abundance was highest. In addition to this, Nagarkar *et al.* (2004) assessed the quality of biofilms nourishment to the grazers from natural intertidal habitats by focusing on the contributions made by the present cyanobacteria. The cyanobacterial species analysed in this study were found to have high protein content and thus, were denoted as important components of marine biofilms enriching the nutritional value to the grazers.

Environmental factors like wave action and insulation have been acknowledged to manipulate the biofilm composition on rocky shores according to field studies by Thompson *et al.* (2005). They examined intertidal biofilms from different shore types (exposed and sheltered) and uncovered that diatoms were about six times more abundant on moderately exposed shore than on sheltered ones. Moreover, the percentage cover of cyanobacteria and the total photosynthesis biomass were greater on exposed shores. Narváez-Zapata *et al.* (2005) also found disparities in the biofilm composition on the shore and implied that this was due to small-scale variations of environmental conditions like wave exposure, which interlinked with the findings by Thompson *et al.* (2005) mentioned above. In theory, dissimilar microspatial habitats on the shore with diverse microbial compositions of the present biofilm can offer a variety of food sources for organisms depending on their position on the shore.

The relevance of diet on the physiology and evolution of marine invertebrates - conclusions

Microorganisms associated with an organism have been recognised to affect the physiology of the host, and especially the role of the symbiotic gut microbiota in digestion and energy uptake is highly valued (Tremaroli and Bäckhed, 2012). In addition to former summarised examples of vertebrates, some other research has been conducted involving certain marine invertebrates and investigating the diversity of their intestinal microbiota with emphasis on the synthesis of critical digestive enzymes by bacteria. One of these studies concentrated on isolated gut bacteria from an abalone species and their ability of to degrade certain plant polysaccharides, and it was interpreted from the results that the processing of food components was supported by gut bacteria which increased the energy yield and metabolism (Erasmus *et al.*, 1997). Diet has been acknowledged as an environmental factor that can shape the microbial community in the intestines of organisms from findings of numerous studies cited previously, and almost all confirm that an alteration in diet provokes a change in the gut microbial composition.

Most studies on the gut microbiota of organisms focus on vertebrates and are established to explore interactions of gut microbiota and host on animals cultured in laboratory conditions. Roeselers *et al.* (2011) addressed the issue of whether a difference in the bacterial composition would be detected between wild and reared animals. For this purpose, they extracted and amplified the DNA from the intestines of zebrafish and contrasted samples from reared and recently caught animals. An overlap of certain bacterial species was found between the two types of zebrafish which was accepted to provide evidence for a core gut microbiota in this species. A core gut microbiota was also discovered by King *et al.* (2012) who analysed and compared

intestinal samples of oysters (*Crassostrea virginica*) collected from different sites. They identified a specific set of gut bacteria which was observed in all samples although they did not originate from the same habitats. Furthermore, the authors uncovered that this core gut microbiome was more similar to ones identified from animals of the same phylum rather than other animal phyla. Such studies matching host-associated microbiomes of distinct phylogenetic groups can give key insights on the coevolution of organisms and their gut microbiota (Sullam *et al.*, 2012).

Since bacteria were proven vital to a range of organisms in the digestion of various dietary constituents, they can have great impact on the metabolism of the host. Subsequently, the presence or absence of important bacteria can modify the fitness of the host likewise, and as fitness is conditional to survival of species, the interactions of host and gut microbiota should not be overlooked when explaining the evolutionary development of a species (Fraune and Bosch, 2010). A fundamental link between gut microbiota and evolution was represented by the display of a change in mating preference in *Drosophila melanogaster* as reviewed earlier (Sharon *et al.*, 2011) and further research needs to be done in order to evaluate whether this could apply to other organisms as well.

The majority of research on the influence of diet on the gut microbiota in animals is based on artificially modified feedstuffs and only few studies actually include natural food resources, in particular of marine invertebrates. Thompson *et al.* (2005) noticed a variation in biofilm composition on rocky shores with somewhat different levels of exposure to environmental factors such as wave action. As their microbial community fluctuates naturally, these biofilms could be suitable representatives of food resources for studies on diet-induced variations in the gut microbiota of intertidal grazers. Many grazing marine invertebrates are dependent on biofilms for nutritional requirements and modified biofilm compositions could evoke an alternation in the gut microbiota of grazing organisms. However, more detailed microbiological analyses of biofilm compositions are necessary steps for the identification of microbial diversity, so that it could be established how different types of biofilms have an impacts on the gut microbiota of grazers in the nature.

In conclusion, it is suggested that future research needs to focus on diet-induced changes in the gut-associated microbiota of marine invertebrates and how this can affect their biology. There is evidence that the gut microbiome of organisms could be a driving force in their evolution and so far, only little attention has been paid on a link between diet and evolution of marine invertebrates. More research needs to integrate such connections so that the evolution of marine invertebrates can be regarded as fully understood.

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