Assembly of Gut Microbial Communities in Freshwater Fish and Their Roles in Fish Condition

YINGHUA ZHA
Abstract

Animal hosts provide associated microorganisms with suitable ecological niches in their intestines. Microbes help their hosts to digest food, protect against pathogens, and influence the host’s metabolisms. Compositional variation of gut microbial communities is common among hosts, and may affect the health status of hosts. Diet and genetic factors are well known to influence the assembly of gut microbial communities. This thesis focuses on disentangling the contributions of factors including host genetics (sex), diet, environment, and other ecological processes to the assembly of gut microbial communities in freshwater fish. The association between gut microbial communities and fish condition is also evaluated in this thesis.

Applying metacommunity theory, we found environmental factors including fish habitat, fish species, their diet, dispersal factors including microbes from fish diet, and ecological drift contributed to the assembly of fish gut microbial communities. The proportion of their contribution varied between fish species, where ecological drift explained more in perch than in roach.

Under natural conditions fish populations face the risk of predation, which can induce competition and impose predation stress within prey individuals. This can therefore lead to changes in their diet qualities and quantities. In this thesis, it was shown that fish diet in terms of qualities and quantities significantly influenced the overall gut microbial composition, and this influence was dependent on fish sex, a host genetic factor. Predation stress was also suggested to significantly decrease the species richness. Furthermore, when fish were experiencing a diet shift, we showed that different bacterial phyla from novel food had different colonization success in the intestine, and this colonization success was positively influenced by predation stress. Fish condition was suggested in this thesis to be affected by gut microbial composition, especially by the contributions of the bacterial phyla Tenericutes and Actinobacteria.

Keywords: freshwater fish, gut microbial communities, predation stress, diet, fish condition, bacterial colonization, metacommunity theory, dispersal

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“To learn makes us realize our deficiency, and to teach makes us know the difficulties. Having realized our deficiency, we may then come to reflect; having known the difficulties, we may be able to strengthen ourselves to overcome them.”

- Confucius, Book of Rites (禮記)

To my family
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


IV Zha, Y., Eiler, A., Johansson, F., Svanbäck, R. (2016) Colonization and extinction probabilities in fish gut microbial communities under diet switch and predation stress (manuscript)

Zha, Yinghua is responsible for the experiment set up/field work, sequencing preparations, data analysis and writing the manuscript draft as first author for Paper I, III and IV. Zha, Yinghua is involved in Paper II with experiment set up, sequencing preparations, and contributed to writing as second author.
In addition to the papers included in this thesis, the author has contributed to the following papers:


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## Abbreviations

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<tr>
<td>16S rRNA</td>
<td>16S Ribosomal Ribonucleic Acid</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>OTU</td>
<td>Operational Taxonomic Unit</td>
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<tr>
<td>NMDS</td>
<td>Nonmetric Multidimensional Scaling</td>
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<tr>
<td>pRDA</td>
<td>Partial redundancy analysis</td>
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<tr>
<td>PLS</td>
<td>Partial least squares regression</td>
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<tr>
<td>βMNTD</td>
<td>β-mean-nearest taxon distance</td>
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<td>βNTI</td>
<td>β-nearest taxon index</td>
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Introduction

Numerous bacteria are present everywhere, in lakes, on the table, on your coffee mug, and of course, on and in you. “You are not human, you are a walking bacterial colony” (Jeroen Raes at TED × Brussels in 2012).

Host associated microbial communities

Microbial communities have been studied in a wide range of environments, such as free-living microbes in freshwater, microbes associated with plants. These studies have aimed, for instance, to investigate the changes in microbial community composition, and the possible functional changes along environmental gradients, such as temperature and salinity. Similar achievements are also needed in the study of symbiotic relationships between animals and the microorganisms inhabiting them (e.g. human, termites, fish) (Ley et al., 2008). Symbiosis refers to a relationship between two different species, in which at least one of them would get benefits from the other but without harming the other (Hooper and Gordon, 2001). In host-microbial relationships, the host supplies habitats and nutrients for the microbes, while the microbes can provide the host with the metabolic products necessary for the host but which the host itself cannot produce.

Studies on fish have found a high number of bacteria, up to $10^8$ cells per gram of the digestive tract, which is much higher than those in the surrounding water (Austin, 2006). This suggested that the gut offers suitable ecological niches for these microorganisms (Cahill, 1990). As symbionts, gut microbiota provide essential functions to their host. The intestine of both humans and fish develop initially in a sterile environment, but completes its maturation with the colonization by microbiota (Fraune and Bosch, 2010), which have been shown to help digest food, protect against pathogens, and influence the metabolism and bioavailability of carbohydrates, sugar and proteins for the host (Dutton and Turnbaugh, 2012). Some researchers consider gut microbiota as an extra organ of the host because its metabolic activities are equal to a virtual organ (Pérez et al., 2010; O’Hara and Shanahan, 2006). Consequently the mutual benefits between a host and its gut microbiota may result in production of metabolites which can contribute to the host’s fitness (Nicholson et al., 2012). The contribution of maintaining host
health is a result of the complex microbial community composition, their functioning and interactions with the host.

Factors affecting the assembly of gut microbial communities

Diet and host genetics

Microbial communities can change quickly as a response to changed environmental conditions. For example, in the incident of the Deepwater Horizon oil spill, microbial community composition increased in relative abundance of microbes capable of degrading hydrocarbons (Hazen et al., 2010). Lozupone et al., (2012) suggested that a similarly fast response could be found in gut microbiota following host diet shifts, where the new substrate induces a selective pressure on the community. Microbes differ in their substrate use, so that niche specialization in gut microbiota leading to changes in bacteria taxa is a consequence of host diet choice (Spor et al., 2011). The phrase “You are what you eat” suggests the importance of nutrition to hosts, and now it has been widely used in gut microbiota studies with a modification to “Your gut microbes are what you eat”, which showed the great interests of host diet as an important factor influencing gut microbiota to study (Ley et al., 2008; Sullam et al., 2012; Wu et al., 2011).

Besides host diet, host genetics have been shown as another factor affecting gut microbiota composition. Stewart et al. (2005) found less similarity in microbial composition in dizygotic compared to monozygotic twins in children. Bolnick et al., (2014a) showed that the variation in gut microbiota in threespine sticklebacks was affected by the Major Histocompatibility class II (MHC) genotypes. In addition, host sex, another genetic trait, has been linked to gut microbial composition (García-Gómez et al., 2013; Markle et al., 2013), and it has been suggested that this link caused by hormone-microbe interactions (Markle et al., 2013; Koren et al., 2012).

Influence of predators

Predators have strong effects on their prey population. Diet quality and quantity are some of the factors affected by predation within organisms in different trophic levels. Increasing predator density, for example, will decrease prey densities, which will increase food resources available for prey and competition among prey. Whereas reduced predation will increase competition among the prey. Increasing predator density may also force some individuals to look for new food resources. So changes in both quantity and quality of food caused by predation would influence the gut microbial communities in prey. Another effect of predation is that it is a common stress
factor for individual prey in nature. Visual and olfactory predator cues have been shown to reduce activities and cause morphological changes in fish, which could help them to reduce predation risk (Brönmark and Miner, 1992; Johansson and Andersson, 2009). Furthermore, hormones released from stress could lead to immunological and behavioral responses in vertebrates, which have been shown to play important roles in regulating gut microbiota communities (Collins and Bercik, 2009; Forsythe et al., 2012; Candela et al., 2012). In general the relationship between trophic levels and gut microbiota may not only be caused by diet differences among trophic levels, it may also be a result of predation stress.

Gut microbiota and host condition

When the environment for the hosts is changed or when the hosts are facing stress, the stable interaction between gut microbiota and hosts will be affected, and this will possibly lead to consequences for host conditions. Gut microbial communities are important for energy acquisition and have been shown to regulate fat storage of their hosts (Turnbaugh et al., 2006; Bäckhed et al., 2014). The acquired energy could be used for growth in length, which is crucial for animals to escape from predator attacks (Lundvall et al., 1999). So it is especially interesting to investigate how gut microbial communities could interact with other environmental factors to affect energy gain for host.

Understanding gut microbial communities in the light of metacommunity theory

Theoretically, gut microbial communities in hosts’ intestines could be considered metacommunities. Metacommunities are defined as multiple local communities that are connected by dispersal of interacting species (Leibold et al., 2004). Metacommunity theory provides four scenarios regarding the assembly of microbial communities depending on the degree of dispersal and the environmental heterogeneity among local environments (Leibold et al., 2004).

Regarding host associated microbial communities, dispersal may be from mother to offspring (Round and Mazmanian, 2009; Dominguez-Bello et al., 2010), from external sources, such as free-living bacteria in the surrounding environment, or from the ingestion of food particles (De Filippo et al., 2010; Koenig et al., 2011; Zhang et al., 2016). Dispersal could cause changes in community composition (Cadotte et al., 2006). However, the dispersal ability of microbes into the intestine varies because of host boundaries or the source types of those microbes, and dictates which pools of microbes associated with the environment or food that could be the sources for gut microbial-
ta communities. After microbes successfully migrate into the intestine, the intestinal environment established by factors such as host genetics, diet, will then determine the outcome of the colonization.
Aims of the Thesis

The overall aims of this thesis are to study how microbes are assembled in freshwater fish intestine, and their contributions to fish condition. More specifically, the studies investigate (1) factors that could regulate the assembly of gut microbial communities, including host genetic variables, environmental factors and other ecological processes, and (2) how microbes in the gut could influence fish condition.

The main focus for each paper includes:

**Paper I** uses metacommunity theory to investigate the contributions of environmental variables and other ecological processes to fish gut microbial community composition, such as ecological drift, or dispersal factors (microbes dispersed from food and surrounding environment).

**Paper II** and **III** investigate the combined effect of predation stress from pike and food quality (Paper II), or food ration (Paper III) on the composition of gut microbial communities and their predicted function.

**Paper IV** investigates how predation stress and host diet shifts due to predation or competition influence the probabilities of colonization and extinction in the host gut microbial community. Specifically, it examines the probabilities of microbes to colonize from the new diet (novel OTUs) into the intestine community and the probability of extinction after a diet switch.

The interactions of gut microbial communities and their host’s condition were analyzed and discussed in **Paper I, II, and III**.
Material and Methods

Field and laboratory experiment
The study in Paper I was conducted at Lake Erken in Sweden during September 4th and 5th, 2013. Perch (Perca fluviatilis) and roach (Rutilus rutilus) were sampled by net fishing from three locations in Lake Erken: littoral nets were located just outside the vegetation at 2 m depth, pelagic nets were set at the surface about 200 m away from the shoreline, and nets for profundal zone were set at 12 meter-depth in the lake adjacent to the pelagic net. All nets were left overnight in the lake and fish were emptied in the following morning. All fish were kept frozen and transported to the lab in Uppsala until further analysis. Potential food resources for fish including microalgae, zooplankton and macro-invertebrates were taken close to the sites where nets were. Water and sediment samples were also sampled to analyze their associating microbes.

The study in Paper II, III and IV was set up with aquaria in the laboratory. Perch were collected from Lake Mälaren in Sweden in May in 2013. Pike (Esox lucius) were collected from Lake Messormen and Hersjön between May and July in 2013. All fish were acclimated to the lab condition at least 4 weeks before the experiment started. During this acclimation period perch were fed frozen chironomids daily and pike were fed perch twice each week. Before the experiment started 18 perch were randomly selected and sacrificed for initial measurements of length, weight, intestine length, sex, and their entire intestine were kept and sampled for the initial measurement of microbial communities used for Paper IV.

To investigate how food quality (different food type) (Paper II), food ration (different food quantity) (Paper III), and the presence of predator affect the gut microbial communities of perch, four perch were put into one aquarium with presence or absence of pike (Figure 1). All perch were fed with either chironomids or Daphnia representing difference in food quality. The amount of chironomids fed to perch was calculated as 5%, 10% and 15% of the average perch body weight. The amount of Daphnia was only fed at the level of 10%. Each treatment was replicated six times. The experiment was run for ten weeks and all perch were sacrificed at the end. Their weight, length, intestine length and sex were recorded for all individuals. We took the entire intestine from each perch and kept frozen until analysis for microbial communities. Chironomids and Daphnia fed to perch and water in the
aquaria were also sampled at the end of the experiment for their microbial communities analysis.

Figure 1. Example of the experiment set up for Paper II, III and IV where perch (in red circle) were with the presence of pike.

Gut microbial communities analysis

Bacterial community composition analysis

Fish gut microbial communities were analyzed by sequencing the V4 region of 16S rRNA genes. Studies have shown that microbial communities in different intestinal segments can vary in their composition (Dhanasiri et al., 2011; Thongaram et al., 2005; Van der Marel et al., 2014; Ringø et al., 2016, 2003). So the entire fish intestine was used in the DNA extraction procedures to avoid the bias of microbial communities if for example only one segment of the intestine was used.

Amplification of 16S rRNA genes was done as described in Sinclair et al (2015) using bacterial primers of 515F and 806R, which is the common primer set used by gut microbiome studies (Marcille et al., 2002; Caporaso et al., 2011; Yan et al., 2016). The amplified genes were sequenced using Illumina MiSeq at National Genomics Infrastructure (NGI) by ScilifeLab in Uppsala, Sweden.

Bacterial community predicted functions analysis

In Paper II and III, PICRUSSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Langille et al., 2013) was used to obtain the predicted functions of bacterial communities by producing the
relative abundance of gene families (gene ontology categories, or GO). MacQIIME (1.9.1.20150604) (Caporaso et al., 2010) was used to get closed reference OTUs at 97% level against the gg_13_5_otus.tar.gz from Greengenes (the 16S rRNA gene database and tools). I followed the workflow suggested by the developers to input the newly picked OTUs into PICRUSt, including normalization by dividing each OTU by the known/predicted 16S copy number abundance, and then calculated the final metagenome functional predictions, which were then categorized with KEGG pathways on level 2.

Fish diet and condition measurement

Short-term and long-term diet analysis

In Paper I, stomach contents were determined as short-term diet. Long-term diet was analyzed using stable isotope ratios of carbon and nitrogen to estimate the trophic level and the proportion of littoral carbon use for each fish.

Stomach contents of each fish were examined under dissecting microscope and were identified to the lowest possible taxonomic group. The length of the prey were measured and converted to biomass for zooplankton based on Bottrell et al. (1976) and for macro-invertebrates using our own length-mass relationships (unpublished). This biomass-based diet was grouped into macro-invertebrates, zooplankton and fish as short-term diet.

Stable isotope analysis of fish muscle tissues was used as a measurement of long-term diet. Isotopic composition of diet is correlated with isotope signals in animals (Fry, 1988). Because of the slow turnover rate of carbon and nitrogen isotope composition in muscle tissues (~2-3 months), isotope analysis is a useful tool for estimating the long-term diet (Alves-Stanley and Worthy, 2009). More specifically, enrichment of δ¹³C could be found in consumers in littoral than in pelagic habitats depending on the different diet sources they choose (France, 1995). For example, consumers in the littoral zone usually use terrestrial detrital carbon. δ¹⁵N is also a robust indicator to estimate the trophic levels in food web for fish (Fry, 1988). Part of the dorsal muscle was taken for δ¹³C and δ¹⁵N measurements, and used in standard formulas to calculate the proportion of littoral carbon use and trophic positions for individual fish (Post, 2002; Matthews et al., 2010).

Fish condition

Fish condition was used in this thesis as a proxy for fish fitness because it is important for fish survival and reproduction (Lundvall et al., 1999). In Paper I and II, fish condition was calculated as mass/length³ (Dutil and
Lambert, 2000). Relative intestine length was calculated as intestine length/fish length in Paper III.

Statistical analyses

Several statistical tools were used in this thesis including both explorative and hypothesis testing. Each method is described in detail in each paper. Here I would like to explain one of the statistical analyses used in Paper I for investigating the assembly of gut microbial communities: null model analysis. This analysis provides a quantitative estimation of the influences of each process to the microbial community assembly, especially for selection, dispersal and drift. Moreover, compared with previous analyses that require predefinition of certain variables that belong to selection or dispersal to affect microbial communities, this null model analysis reversed the procedure by using ecological patterns to identify which variables could impose selection or dispersal (Stegen et al., 2013). The analysis was done as described by Chase et al. (2011), Stegen et al. (2012, 2013), Langenheder et al. (2016) and Yan et al. (2016), and through personal communications with Qingyun Yan.

Within metacommunities, the turnover of the community composition is primarily determined by a combined effect of selection, dispersal and drift (Vellend, 2010). The contribution of each process varies among systems but phylogenetic turnover can be used to infer which ecological processes are important for community assembly in a certain system. In this analysis, closely related species are assumed to have more similar habitat preferences than those who are distantly related (Losos, 2008). Phylogenetic turnover is calculated as the $\beta$-mean-nearest taxon distance ($\beta$MNTD) between pairs of microbial communities (Fine and Kembel, 2011). $\beta$MNTD is then compared with a null model, which is randomly shuffled across the tips of the phylogeny, and the differences between the models is referred as the $\beta$-nearest taxon index ($\beta$NTI), which indicates whether pair of communities is determined by environmental selection (Stegen et al., 2013).

A $|\beta$NTI| < 2 indicates that the microbial communities are not determined by environment selection (Yan et al., 2016; Stegen et al., 2013). I further used the Raup-Crick metric based on Bray-Curtis distance using OTU relative abundance ($\text{RC}_{\text{bray}}$) to decipher how these communities were assembled (Chase et al., 2011; Stegen et al., 2013, 2012). According to Chase et al. (2011), -0.95 < $\text{RC}_{\text{bray}}$ < +0.95 indicates that the community was significantly decided by drift, while $\text{RC}_{\text{bray}}$ < -0.95 indicates that the community was more similar than expected by drift because of mass effects, while $\text{RC}_{\text{bray}}$ > +0.95 indicates that the community was less similar than expected by drift due to dispersal limitation (Stegen et al., 2013; Langenheder et al., 2016).
Results and Discussions

Factors influencing the assembly of gut microbial communities

**Paper I** investigated the assembly of the gut microbial communities in two freshwater fish species (perch and roach), where local environmental conditions, ecological processes and dispersal are considered to be important in determining the variations in community composition (Lindström and Langenheder, 2012; Vellend, 2010). Using a null model (details in materials and methods), I found that both environmental selection and ecological processes played roles in community assembly. More specifically, in perch (Figure 2A), ecological drift was found to be the major factor explaining variation (60%), whereas environmental selection explained 15%. While in roach (Figure 2B), environmental selection explained 37% of the variation in community composition, followed by ecological drift, dispersal limitation and mass effect.

![Figure 2](image_url)

*Figure 2.* Proportions of the contribution of environmental and ecological processes to the variance of microbial communities among individual perch (A) and roach (B) as determined by statistical models.

Further, environmental selection and dispersal factors were analyzed into subsections for more information. Source Tracker analysis, which was used to predict the source of microbial communities in a set of microbial “sink” samples (Knights *et al.*, 2011), identified that microbes associated with the diet were an important potential dispersal source for gut microbiota whereas microbes from surrounding water and sediment were not important. As shown by partial redundancy analysis (pRDA) (Figure 3A), and partial least square analysis (PLS), environmental selection, including roach host fish species, profundal habitat, fish length, trophic position and macro-invertebrate diet significantly covaried with gut microbial communities.
Dispersal factors including microbes from algae, macro-invertebrates and zooplankton were also revealed to correlate with the gut microbial communities (Figure 3B). In fact, dispersal factors explained a greater share of the variation compared to environmental factors, although the shared contribution by these two types of factors was greater and the greatest part was unexplained variation (Figure 3C).

Figure 3. pRDA plot showing the covariation between the composition of gut microbial communities and environmental selection (A), and dispersal factors (B) shown as arrows. The proportion of variance in gut microbial communities explained by environmental selection and dispersal factors determined by variance partitioning analysis (C).

Many studies have investigated possible factors in determining the assembly of gut microbial communities in animals. One of the most studied models is zebra fish. For example, in the zebrafish raised under conventional laboratory conditions, Burns et al. (2016) suggested that neutral processes are enough to explain a large part of the variation in the gut microbial communities. While Yan et al. (2012) and Yan et al. (2016) who showed that both the gut environment and some of the selective factors during the host development (from early to adult stages) are important in shaping the fish gut microbial communities. In roach, environmental selection was found to largely
influence gut community composition (Paper I). Furthermore, the distinctive difference between gut microbiota communities and microbial communities in the surrounding water indicates that the local environment (individual fish) has a great importance for microbial community assembly. At the same time, the significant correlation between microbial community composition with factors such as fish habitat and trophic positions could directly or indirectly influence the gut environment. This seems to fit the species sorting perspective in the metacommunity theory, which assumes that the dispersal is high enough for immigration but the local habitat conditions define the community composition (Lindström and Langenheder, 2012). Thus, in the case of fish gut microbial communities, microbes in the surrounding water and in food resources continuously pass through the fish body. However, each fish experiences a different environment, such as different habitats and different food resources, which could possibly lead to heterogeneity in their intestines, so that gut microbial communities would be selected based on the difference in the intestinal environment. However, the role of ecological drift in shaping the assembly of gut microbial communities was also large, especially in perch (Paper I). This seems to corroborate results from other studies suggesting ecological drift as an important factor influencing gut microbial composition (Burns et al., 2016).

The different contributions of environmental and ecological processes to the variance in perch and roach gut microbial communities could also suggest some other factors that shape the gut microbiota. One possible and obvious factor is the genetic differences between perch and roach, which previously has been found to influence gut microbial communities (Macfarlane and Macfarlane, 2009; Benson et al., 2010; Brooks et al., 2016; Koenig et al., 2011; Bolnick et al., 2014a). Another possible explanation could be the difference of the digestive structure between perch and roach. Food passes through the stomach before it enters the intestine in perch, while roach have no stomach so food directly goes into the intestine. And pH influence in the stomach could be an important factor to affect gut microbial communities as it is for free-living microbes. However these ideas remain speculations and need further investigation.

In aquaria experiments, I found that both the type of diet (quality) and the amount of food eaten (quantity) influenced gut microbiota (Paper II and III). In Paper II, both α- and phylogenetic diversity were significantly affected by the interaction of diet quality and sex, which was coherent with a previous study by Bolnick et al (2014b). But the significant influence of the interaction between diet quality and predation stress on β-diversity of the gut microbial communities among perch has not been shown before. The effect of stress and diet quality interaction on community composition led to a shift in roughly half of predicted microbial community functional pathways (23 out of 41 KEGG pathways were affected by a stress-diet interaction) (Paper II). In Paper III where perch were fed with three quantity levels of chiron-
omids, I found the interaction of food ration and sex also affected estimated total species richness, Chao1 (Figure 4). Food ration also influenced the β-diversity of microbial communities. Similar to the effect of diet type, food ration levels also affected several functional categories involved in biosynthesis of other secondary metabolites, metabolism of cofactors and vitamins, and digestive system.

![Figure 4](image)

**Figure 4.** Gut microbial community changes in Chao 1 responding to the interaction of food ration and sex.

Host sex, another genetic trait, has been correlated to gut microbial composition (García-Gómez *et al.*, 2013; Markle *et al.*, 2013; Bolnick *et al.*, 2014b). The mechanisms behind the interaction between food and sex affecting microbial diversity found in Paper II and III is still not clear. One plausible mechanism is that sex hormones influence the effect of food on gut microbiota. Sex hormones have been shown to play an important role in regulating bacterial metabolism and growth (Freire *et al.*, 2011). For example, Markle *et al.* (2013) showed that gut microbial composition in mice developed sex specific difference in puberty. However, we need further investigations to
test whether the male and female sex hormones affect bacterial growth differently when they are provided different amounts of food.

Effect of predator presence on the assembly of gut microbial community and their colonization and probability of extinction

Pike presence also had an effect on the gut microbial community in perch (Paper II, III and IV). In both Paper II and III, I found that α diversity decreased in the presence of pike. Firmicutes and Fusobacteria also seemed to have a higher number of opportunities to respond to pike presence/absence than the other phyla. Furthermore, pike presence had effects on several predicted functional categories as well, suggesting that stress could affect the function of the gut microbiota. Interestingly, when perch switch diet, the presence of pike positively influenced the probability of novel OTUs from the new food to colonize the intestine (Paper IV). This was especially seen in OTUs belonging to Firmicutes (Figure 5). For the probability of extinction, I found that OTUs present in the water column had a lower extinction probability in the intestine than the OTUs not found in water.

Previous studies on human and other animals have shown that stress can affect the gut microbial communities (Collins and Bercik, 2009; Forsythe et al., 2012; Candela et al., 2012). For example, Bailey et al. (2011) showed that social disruption stressor could affect the gut microbiota in mice. The studies present in this thesis (Paper II, III and IV) show that stress induced by risk of predation can also affect gut microbial communities. The stress responses in teleost fish are similar to any other terrestrial vertebrate (Wendelaar, 1997). The primary response of fish to stress is to release stress hormones into circulations together with an endocrine response, which is strongly related to fish immune systems (Wendelaar, 1997). Several studies have shown that both innate and adaptive immune systems can affect gut microbiota composition and diversity (Vijay-kumar et al., 2010; Zhang et al., 2015). Thus, the physiological changes a fish host facing predation stress could result in environmental changes for their gut microbes, which in turn could change the composition or functioning of the microbial communities (Collins and Bercik, 2009).

The success of colonization by novel OTUs might occur because stress in general reduces gut microbial diversity leaving open niches, which in turn facilitates colonization opportunities. Alternatively, stress might influence the host immune system, which could regulate colonization of microbes (O’Mahony et al., 2009; Bailey, 2012). Microbes in water are constantly ingested by fish and could potentially serve as a re-colonization source for
the gut microbial communities. OTUs from the water column had a lower probability of extinction in intestine, which might provide a rescuing effect for the gut microbial communities. This is suggested to happen mainly in communities that experienced stress and is connected with dispersal (Fussmann and Gonzalez, 2013; Low-Décarie et al., 2015).

![Figure 5. The effects of predation on the colonization probability in fish intestine by phylum Firmicutes (paired t-test).](image)

Role of gut microbial communities in fish condition

In natural populations, increasing body size helps hosts to reduce vulnerability to predator attacks, and energy storage could reduce starvation mortality of hosts. Turnbaugh et al. (2006) and Bäckhed et al. (2014) have shown that gut microbiota could affect host fitness or the trade-off between energy storage and growth. In this thesis, gut microbial communities affected the host condition (Paper I and II). In Paper I, I show that the importance of dispersal of microbes was related to fish condition for both perch and roach. However, microbes from different sources appeared to contribute to fish condition in different degrees. For example, dispersal of microbes from macro-invertebrates was positively related to fish condition in both perch and roach,
while microbes from zooplankton were negatively related to fish condition (Figure 6). When fish were fed the same amount of macro-invertebrates and zooplankton, macro-invertebrates could lead to a higher growth in perch (Borcherding et al., 2007). Ahlgren et al. (1997) suggested that macro-invertebrates could access better quality food during the late summer and autumn in Lake Erken, which might result in that macro-invertebrates are a diet of better quality for perch. The results found in Paper I suggest that the food-associated microbiota might also, besides the quality of the diet, affect host fitness.

**Figure 6.** Circles of correlations from PLS regression analysis. Fish condition was response variable (orange lines), and dispersal factors were predictor variables (blue lines) in perch (A) and roach (B).

The length of juvenile fish is important for them to escape from predator attacks (Lundvall et al., 1999). And fish condition is related to the resistance they could have for going through harsh situations for example periods of starvation (Dutil and Lambert, 2000; Bender et al., 2008). In Paper II, I found that the relative abundance of Tenericutes was negatively related to perch length, especially in the presence of pike (Figure 7A). Furthermore, I show that the relative abundances of Tenericutes and Actinobacteria were related to the length of the individuals, implying that an overall positive effect of Tenericutes on host length can be found at higher relative abundances of Actinobacteria (Figure 7B). Fish body condition was also related to the relative abundance of Tenericutes and Actinobacteria (Figure 7C). Individuals with a high contribution of both Tenericutes and Actinobacteria had a poor condition, which was opposite to the effect on fish length. This suggested that young fish might trade off investing the energy gain between increase in length versus condition. It also suggests that gut microbiota may
influence the trade-off between important mortality factors in natural populations.

*Figure 7.* Microbiota effects on fitness components in perch. A) Effects of proportion *Tenericutes* at the end of the experiment on length in the presence (green) and absence (orange) of pike. The interaction between relative abundances of *Tenericutes* and *Actinobacteria* on both B) length and C) condition at the end of the experiment. Color scales represents length (B) and condition (C) of the perch.
Conclusions and Future Perspectives

In this thesis, these major conclusions can be drawn:

- Both environmental and ecological processes, including dispersal and ecological drift, could influence the assembly of gut microbial communities in freshwater fish.
- Both diet quantity and quality could influence the composition and diversity of gut microbial communities as well as their predicted functions.
- The effect of diet on the composition and diversity of the gut microbiota is dependent on host sex.
- Predation stress decreases the diversity in gut microbial communities and affects their composition and predicted function.
- Gut microbial communities might affect fish fitness in terms of condition and growth.

This thesis strengthens the knowledge of the assembly of gut microbial communities in animals other than humans. Fish models provide good tools for investigating gut microbiota, and could possibly be used as models to extend the obtained knowledge to human studies. It is evident from my thesis that the factors influencing the assembly of gut microbiota are of importance to investigate not only how microbes settle and develop in host intestine, but also about their crucial roles in regulating and affecting host condition. This is important especially to understand how perturbation might lead to dysbiosis in hosts.

Gut microbial communities are present in rather complex environments. Not only could the host itself affect the communities, other factors that affect the host could also indirectly influence its inhabiting microbial communities. It will be interesting to investigate more possible factors to affect gut microbial communities. For example, how does switching the host diet, especially the frequency of those switches, affect the microbial composition? Also the effects of gut pH and bacterial predation on gut microbial communities are poorly understood. Evaluating those potential variables could contribute to explaining the complex assembly of microbial community composition.

The role of microbes in host fitness is also of great interest to continue studying in different groups of organisms like fish and mammals. The suggested interactions between individual host and their bacterial phyla present-
ed in this thesis provided some basic but interesting results, such as the effect of relative abundance of *Tenericutes* and *Actinobacteria* on fish condition. A high fish condition was attained when one of the phyla had a high relative abundance while the other one was low. Similar results have been obtained from human microbiome studies, where *Firmicutes* to *Bacteroidetes* ratio have been related to obesity (Chakraborti, 2015; Ley *et al.*, 2006; Kasai *et al.*, 2015). It will be interesting to see if the ratio between *Tenericutes* and *Actinobacteria* relating to fish condition exists in other species, and whether it is similar for fish host in different environments. If this is the case, then this ratio could be used as an indicator to evaluate host fitness. Moreover, *Fusobacteria* was shown to positively react to pike predation stress in this thesis. It would be interesting and useful to further investigate the underlying mechanisms behind this reaction.
Tarmar från de flesta djur, inklusive människa och fisk, utvecklas initialt i en steril miljö. Vid kontakt med omgivande miljö, och senare även födointag, vidareutvecklas tarmarna med koloniseringen av mikroorganismer. Dessa mikrober hjälper sin värd att smälta födan, skydda mot sjukdomar, och syntetisera näringsämnen som värdens inte kan producera, som till exempel vitamin B. Värden i sin tur tillhandahåller föda till mikroorganismerna. Interaktionen mellan värd och deras tarmflora bidrar till värdens hälsa som ett resultat av den komplexa sammansättningen av mikrobsamhället och deras funktion. Tarmfloran är divers och kan skilja mycket mellan individer. Vilka mekanismer som påverkar sammansättningen av tarmfloran och hur dessa faktorer påverkar diversiteten och skillnader mellan värden är därför viktigt att undersöka.

Studier, främst på människor, har visat att tarmfloran är ganska plastisk och kan påverkas av värdens genetiska uppsättning, kön och särskilt av värdens diet. Studier på människa och däggdjur har funnit att mikrobiella samhällen i tarmen kan grupperas med diet där det är stor skillnad mellan rovdjur, allätare och växtätare. Liknande, fritt levande bakteriesamhällen i till exempel vatten kan snabbt förändras som respons på förändrade miljöförhållanden. Till exempel, efter ett oljeutsläpp i Mexikanska golfen förändrades bakteriesamhället till ett som mer effektivt bröt ner kolväten, vilket tyder på att förändrade substrat för mikrober har en stark selektiv påverkan på den mikrobiella sammansättningen. Detta gäller även tarmens mikrobiella samhällen där substraten påverkas av värdens födointag.

hos bytesorganisationer, vilket har visat sig spela en viktig roll för påverkan på tarmflorans sammansättning.

Syftet med den här avhandlingen är att i fält och laboratorieexperiment undersöka hur tarmfloran hos sötvattensfisk påverkas av olika miljöfaktorer. Speciellt är jag intresserad av hur stress från predation samt födans kvalitet och kvantitet påverkar tarmflorans sammansättning. Genom att använda klassisk ekologisk teori från meta-samhällsekologin, studerade jag även närmare vilka ekologiska processer som bidrog till sammansättningen av tarmfloran.


Samspelet mellan tarmfloran och fiskens energiintag i form av tillväxt och kroppsmassa utvärderades också i studierna. Här hittade jag att mikrober från olika födokällor bidrog till individens energiintag. Mikrober från bottenlevande djur bidrog positivt till både abborrens och mörtens energiintag, medan mikrober från djurplankton var negativt relaterat till fiskens energiintag. Bottenlevande djur har som föda visats vara en bättre näringskälla jämfört med djurplankton, så den positiva effekten på fiskens energiintag när den äter bottenlevande djur kan vara en kombination av födans kvalitet och
动物的消化道是在无菌的环境中发育起来的。从分娩时开始接触在母亲产道中的微生物，随着开始进食，食物空气中的微生物，也开始在新生儿肠道中建立。伴随着婴儿的食物渐渐开始接近成年人的食物，肠道中的微生物群落结构会逐渐趋向于稳定，最终形成成熟的肠道菌群。动物消化道以及食物为这些菌群提供了合适的生活条件，而这些微生物也可以帮助消化食物，抵御病原的入侵，还可以合成出一些人体自身无法合成的物质，比如维生素B。所以肠道菌群（共生体）和动物（宿主）之间构成了共生关系。很多科学家将肠道菌群称之为另一个人体器官，并且与人体健康息息相关。

肠道菌群的结构非常复杂，动物个体之间的差异也非常大。大多数在人体的研究显示肠道菌群对于宿主和环境因素有很强的可塑性，比如宿主的基因、性别，尤其是食物。人体和许多哺乳动物的研究指出肠道菌群根据食物的种类具有很强的聚集性。这个和非共生的菌类具有相似性。研究发现，在墨西哥湾石油泄漏事件中，水中的微生物群落可以很快地变换自身的微生物组成去分解碳氢化合物，表明改变的基质对于微生物具有选择作用。所以这个也可以应用在共生关系的微生物群落研究中，对于这些微生物，宿主的食物就是他们可以利用的基质。

对于野生动植物，比如鱼类，也同样经历着相似甚至是更为严苛的生存环境。这也就导致了也许更为复杂的肠道菌群及其宿主之间的关系。处在食物链更高级别的鱼类会捕食更低级别的鱼类，这样会导致低级别的鱼类去选择不同种类以及不同数量的食物。捕食鱼类数量的增加会减少被捕食鱼类的数量，这样也间接地降低了被捕食鱼类之间的竞争，他们也会有更多的食物。另一方面，捕食鱼类数量的减少会增加被捕食鱼类之间对于食物的竞争，这样会导致他们食物的减少或者是去寻找新的食物。除了食物数量和种类的变化，捕食时面对的压力也是鱼类在食物链中会经历的。这种压力会引发捕食鱼类减少他们的活动来躲避捕食，同时他们体内的激素也会发生变化，而激素也是影响肠道菌群的重要因素之一。
我们通过结合野外作业与实验去探讨淡水鱼类的肠道菌群是怎样建立的，同时捕食压力，食物数量及种类的改变会怎么样影响它们肠道菌群的结构。继而我们应用 meta-群落假说(meta-community theory)去研究具体的环境因素及生态过程会对肠道菌群的建立产生影响。

我们发现和鲈鱼(Perca fluviatilis)相比，环境选择是决定拟鲤(Rutilus rutilus)肠道菌群的重要因素之一。而生态漂变(ecological drift)则解释了鲈鱼肠道菌群中超过一半的变异。接着我们将环境选择具体化，发现鱼的种类，他们的栖息地以及营养级都显著地影响了肠道菌群的结构。同时，我们发现来自于浮游生物，湖底的无脊椎动物(鱼类的食物)的微生物是鱼类肠道菌落中的重要组成。在另一个实验中，鲈鱼与其捕食者白斑狗鱼(Esox lucius)共同生活在鱼缸中，我们借此来研究白斑狗鱼对于鲈鱼的捕食压力是否会影响鲈鱼的肠道菌群。同时我们喂鲈鱼不同重量的摇蚊幼虫或者水蚤来检测不同重量和不同种类的食物对于菌群的影响。我们发现白斑狗鱼的捕食压力显著地降低了鲈鱼肠道菌群的多样性。这有可能是由于当鲈鱼面对捕食压力时，其体内的生理变化导致微生物生存的环境也发生了改变，从而间接的改变了菌群的结构。更为有趣的是，我们发现在应对捕食压力的鲈鱼中，厚壁菌门(Firmicutes)可以更好的在肠道中建立。这一点有可能是由于在面对外界扰乱时，宿主肠道中会产生成空的生态栖息地，这些空的栖息地可以被伴随着外界食物进入的新的微生物利用。我们同样发现食物对于肠道菌群的重要影响。具体来讲，我们发现菌群的多样性和食物数量以及宿主的性别相关。也就是说雄性和雌性鱼类对于食物数量的增加有着不同的反应。

另外，我们同时发现来自于不同食物的微生物对于鱼类身体状况的影响也是不一样的。来自于无脊椎动物的微生物对鱼类身体状况有着积极的影响，而来自于浮游生物的微生物则会降低鱼类身体状况。研究表明和浮游生物相比，无脊椎动物是一种更好的鱼类食物。所以我们这里发现的对鱼类身体状况的积极影响有可能是无脊椎动物单纯作为食物以及它们所附属的微生物的共同作用。我们同样发现硬壁菌门(Tenericutes)和放线菌门(Actinobacteria)之间的比例和鱼类的身体状况同样有关系。对于野生动物，好的身体条件可以帮助它们躲避捕食和渡过类似于饥饿的状况。所以我们研究中发现的肠道菌群和鱼类身体状况之间的关系显示了肠道菌群对于宿主生态和进化的重要作用。
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