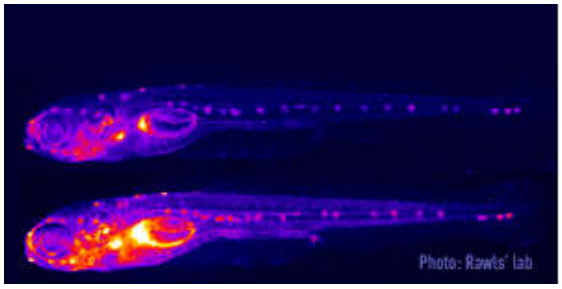

1st International
Fish - Microbiota Workshop

Trondheim, 19th – 21st June 2017

NTNU
Department of Biotechnology
and Food Science

NTNU Biotechnology
The enabling technology programme 2013-2020

NTNU OCEANS

The First International Fish – Microbiota Workshop

Trondheim, June 19th – 21st 2017

Program

Monday 19th of June: Functionality of host-microbe interactions

08:30 – 09:15	Registration/coffee
09:15 – 09:30	Welcome
09:30 – 10:30	Talks (Chair: John Rawls)
	Peter Bossier, Sylvia Brugman, Chao Ran
10:30 – 11:00	Coffee break
11:00 – 12:00	Talks (Chair: Kat Milligan-Myhre)
	Zhen-Yu Du, Colin Lickwar, John Rawls
12:00 – 13:15	Lunch
13:15 – 14:35	Talks (Chair: Peter Bossier)
	Zhen Zhang, Mickaël Castelein, Kat Milligan-Myhre, Zhigang Zhou
14:35 – 15:00|	Coffee break
15:00 – 17:00	Discussions on axenic fish model systems (John Rawls)
17:30 –	Reception at campus

Tuesday 20th of June: Community assembly in fish

09:00 – 10:20	Talks (Chair: Adam Burns)
	Brendan Bohannan, Konstantinos Kormas, Trond Kortner, Jaime Romero
10:20 – 10:50	Coffee break
10:50 – 11:50	Talks (Chair: Brandon Schlomann)
	Nicolas Derome, Ingrid Bakke, Martin Llewellyn
12:00 – 13:00	Lunch
13:00 – 14:00	Talks (Chair: Brendan Bohannan)
	Brandon Schlomann, Sol Gomez de la Torre Canny, Adam Burns
14:00 – 15:00	Poster session with coffee/snacks
15:00 – 17:00	Discussions on microbial community assembly in fishes (Brendan Bohannan)
19:30 –	Workshop dinner at Munkholmen Island

Wednesday 21st of June: Microbial control in aquaculture

09:00 – 10:20	Talks (Chair: Ragnhild Vestrum)
	David Huyben, Bastian Barker Rasmussen, Pavlos Makridis, Marc Verdegem
10:20 – 10:50	Coffee break
10:50 – 11:50	Talks (Chair: Bastian Barker Rasmussen)
	Olav Vadstein, Kari Attramadal, Meiling Zhang
12:00 – 13:00	Lunch
13:00 – 13:40	Talks (Chair: Olav Vadstein)
	Ragnhild I. Vestrum, Mark Liles
13:50 – 15:00	Discussions on aquaculture applications with coffee/snacks (Olav Vadstein)
15:00 – 15:30	Closing remarks
18:00 –	Outdoor social activity

Arrangement committee:
Olav Vadstein and Ingrid Bakke, NTNU Norwegian University of Science and Technology
Brendan Bohannan, University of Oregon
John Rawls, Duke University

Table of Content

Functionality of host-microbe interactions

Community assembly in fish

Microbial control in aquaculture

Mild stress is beneficial in host microbial interactions: within generation and transgenerational experiments

Prof peter bossier[1], Dr Kartik Baruah[1], Dr Parisa Norouzitallab[1]
[1]Ghent University, Lab Aquaculture & Artemia Reference Center, Ghent, Belgium

The gnotobiotic Artemia test system represents an exceptional system for carrying out studies on the interference of microbial communities and/or specific (pathogenic) strains. We have previously shown that subsequent to a non-lethal heat shock, axenic Artemia nauplii become resistant to a lethal heat shock and Vibrio infection, suggesting that there is a metabolic link between abiotic and biotic stress. Inducing pathogen resistance through a non-lethal heat shock is not practical.
Pyrogallol pretreament induced protective effects in the Artemia against V. harveyi infection. By pretreating Artemia with pyrogallol in the presence or absence of an antioxidant enzyme mixture, we showed that the Vibrio-protective effect of the compound was caused by its prooxidant action. We further showed that the generation of prooxidants is linked to the induction of heat shock protein Hsp72, which is involved in eliciting the prophenoloxidase and transglutaminase immune responses. Also carvacrol was shown to be a potent HSP72 inducer. Induction of HSP72 was associated Vibrio harveyi resistance. The initial generation of reactive molecule H2O2 by the compound plays a key role. The idea that phenotypic traits are heritable remains under debate. Daily exposure to a non-lethal heat shock of the parental population increased tolerance towards lethal heat stress in three subsequent unexposed generations using parthenogenetic Artemia (originating from one single female) as observed in a common garden experiments. This increased thermotolerance was associated with increased Vibrio resistance. This transgenerational inheritance of the acquired traits was associated with altered levels of global DNA methylation and acetylated histones (H3, H4) in the heat-shocked group compared to the control group. The results suggest that immune responses in invertebrates can be trained, and epigenetic reprogramming of (selected) immune effectors is likely to have a central place in the mechanisms leading to trained immunity.

Effect of microbial composition on severity of intestinal inflammation in zebrafish

Martijn Vos[1], **Dr. Sylvia Brugman[1]**, Dr. Maria Forlenza[1], Prof.Dr. Michiel Kleerebezem[1], Prof. Dr. Geert Wiegertjes[1]
[1]Wageningen University, Wageningen, Netherlands

Dysbiosis of the intestinal microbial community is considered a risk factor for the development of chronic intestinal inflammation as well as other diseases such as diabetes, obesity and even cancer. Study of the innate and adaptive immune pathways controlling bacterial colonization has however proven difficult in rodents, considering the extensive cross-talk between bacteria and innate and adaptive immunity and difficult access. Here, we used the zebrafish to study dysbiosis. First, we investigated whether disturbance of the microbiota through antibiotic treatment resulted in alteration of the severity of chemically-induced intestinal inflammation. Indeed, a microbiota dominated by Fusobacterium (Cetobacterium somerae) reduced neutrophil recruitment and damage upon chemically-induced colitis. In further studies we investigated the effect of adaptive immune development on the microbial composition. We find that some species (known pathobionts such as Vibrionales) are are effectively suppressed by adaptive immune cells. Using cell transfer experiments, we confirmed that adoptive transfer of T lymphocytes, but not B lymphocytes into Rag1-deficient recipients suppresses outgrowth of Vibrionales. Interestingly, this transfer of T lymphocytes also resulted in the upregulation of chemokines produced by epithelial cells. With this zebrafish model we will be able to understand the more fundamental processes underlying host-bacterial cross-talk in during both larval (only innate immunity) and adult stages of life.

Thymol and Carvacrol Affect Hybrid Tilapia through the Combination of Direct Stimulation and an Intestinal Microbiota-Mediated Effect: Insights from a Germ-Free Zebrafish Model

Chao Ran[1], Jun Hu[1], Wenshu Liu[1], Zhi Liu[1], Suxu He[1], Bui Chau Truc Dan[2], Nguyen Ngoc Diem[2], Ei Lin Ooi[2], Zhigang Zhou[1]
[1]Feed Research Institute, Chinese Academy of Agricultural Sciences, China, [2]Novus Aqua Research Center, Novus International, Inc., , USA

Essential oils (EOs) are commonly used as animal feed additives. Information is lacking on the mechanisms driving the beneficial effects of essential oils in animals, especially the role played by the intestinal microbiota of the host. In this study, we investigated the relative contribution of direct effect of essential oils and indirect effect mediated by the intestinal microbiota, by using a germ-free zebrafish model. Juvenile hybrid tilapia was fed control diet or diets containing 60-800 mg/kg of an EO product Next Enhance® 150 (NE) containing equal levels of thymol and carvacrol. The key humoral and cellular innate immune parameters were evaluated after the feeding. The gut microbiota of tilapia fed with control or NE diet (200 mg/kg) for 2 weeks was transferred to 3-dpf GF zebrafish, and the expression of genes involved in innate immunity and tight junctions was evaluated at 6-dpf. NE was also directly applied to GF zebrafish to test the direct effect of NE. Results showed that NE supplementation at 200 mg/kg enhanced phagocytosis activity of head kidney macrophages and plasma lysozyme activity of tilapia compared with the control, indicating an immunostimulatory effect. Compared with those colonized with control microbiota, GF zebrafish colonized with NE microbiota showed attenuated induction of immune response marker genes saa, il1β, and il8. NE treatment of GF zebrafish at 2 and 20 mg/L for 1 day up-regulated the expression of il1β and claudin1, while at day 3 the expression of occludin2 was higher in the 0.2 mg NE/L group versus GF control. In conclusion, NE may affect the immunity of tilapia through a combination of factors, i.e., direct effect on host tissue (immune-stimulating) and indirect effect mediated by microbial changes (immune-relieving), with the direct stimulation effect as the mainstream, which counteracted the microbiota-mediated effect.

Establishment of germ-free zebrafish model and its application in fish lipid metabolism study

Yu-Hong Sun[1], Yu-Kun Liu[1], Yi Sheng[1], **Prof. Zhen-Yu Du[1]**, Prof. Mei-Ling Zhang[1]
[1]*Laboratory of Aquaculture Nutrition and Environmental Health (LANEH), East China Normal University, Shanghai, China*

In mammals, gut microbiota participated in the lipid metabolism. It has been proved that intestinal bacteria in zebrafish can regulate the intestinal absorption and metabolism of fatty acids, and different bacteria show different performance in the lipid droplets formation. However, the exact role of intestinal microbiota in lipid metabolism in fish remains unclear. Germ-free mice have been widely used in the research of lipid metabolism in mammals, while application of germ-free zebrafish (GF zebrafish) in the lipid metabolism research has just begun. Our lab constructed GF zebrafish by washing fertilized eggs with antibiotics. The hatched juvenile fish were reared in gnotobiotic zebrafish medium and the sterility was checked every day by bacterial culturing tests. The role of commensals in lipid absorption was studied. Germ-free and conventional zebrafish were fed with egg yolk and two hours later, zebrafish were collected to detect the quantity and size of lipid droplet in the intestinal epithelial by Transmission Electron Microscope (TEM). The results indicated that lipid droplets in the intestinal epithelial of GF zebrafish were fewer than those in conventional zebrafish. Unsurprisingly, zebrafish fed with egg yolk have more lipid droplets than unfed ones in both germ-free and conventional groups. RNAseq and real-time PCR were applied to detect the gene expression level in these groups and we found that lipid metabolism was more active in conventional zebrafish, including triglyceride synthesis, transfer and lipolysis. Expression level of genes related to triglyceride transfer increased significantly in conventional zebrafish fed with food, suggesting intestinal microbiota play an important role in this process. In summary, we successfully established GF zebrafish and verified that the lipid transfer is largely dependent on the existence of intestinal microbiota, thus our model could be a powerful platform for the future study of fish lipid metabolism.

Genomic dissection of conserved transcriptional regulation in intestinal epithelial cells

Colin Lickwar[1], Gray Camp[2,3], Matthew Weiser[2], David Kingsley[3], Terrence Furey[2], Shehzad Sheikh[2], John Rawls[1,2]
[1]Duke University, Durham, United States, [2]University of North Carolina Chapel Hill, Chapel Hill, United States, [3]Stanford University, Stanford, United States

The intestinal epithelium is a primary interface for host-microbe interactions, serving to maintain microbial homeostasis while absorbing dietary nutrients. However, a comprehensive understanding of the conserved biology and underlying transcriptional regulatory mechanisms that are common across vertebrate species is unknown. We generated genome-wide mRNA and accessible chromatin data from adult intestinal epithelial cells (IECs) in zebrafish, stickleback, mouse, and human to determine if conserved IEC functions are achieved through common transcriptional regulation. We found evidence for substantial common regulation and conservation of gene expression regionally along the length of the intestine from fish to mammals, and identified a core set of genes comprising a vertebrate IEC signature. We also identified transcriptional start sites and other putative regulatory regions that are specifically accessible in IECs in all four species. Although, these sites rarely showed sequence conservation from fish to mammals, surprisingly, they drove highly conserved IEC expression in a zebrafish reporter assay. Common transcription factor binding sites (TFBS) found at these sites in multiple species indicate that sequence conservation alone is insufficient to identify much of the functionally conserved IEC regulatory information. Among the rare highly sequence-conserved IEC-specific regulatory regions, we discovered an ancient enhancer upstream from her6/HES1 that is active in a distinct population of Notch-positive cells in the intestinal epithelium. Together, these results show how combining accessible chromatin and mRNA datasets with TFBS prediction and in vivo reporter assays can reveal tissue-specific regulatory information conserved across 420 million years of vertebrate evolution. We define an IEC transcriptional regulatory network that is shared between fish and mammals, and establish an experimental platform for studying how evolutionarily distilled regulatory information commonly controls IEC biology and the response to microbes.

Microbiota regulate intestinal epithelial gene expression by suppressing the transcription factor Hepatocyte nuclear receptor 4 alpha'

James M. Davison[1,2], Colin R. Lickwar[1], Lingyun Song[3], Ghislain Breton[4], Gregory E. Crawford[3], **John Rawls[1]**

[1]Department of Molecular Genetics and Microbiology, Center for the Genomics of Microbial Systems, Duke University, , United States, [2]Department of Cell Biology and Physiology, University of North Carolina, , United States, [3]Department of Pediatrics, Division of Medical Genetics, Center for Genomic and Computational Biology, Duke University, , United States, [4]Department of Integrative Biology and Pharmacology, McGovern Medical School, Texas, United States

Microbiota influence diverse aspects of intestinal physiology and disease in part by controlling tissue-specific transcription of host genes. Many mouse genes that are transcriptionally regulated by microbiota have zebrafish homologs that are similarly responsive, suggesting the existence of evolutionarily conserved regulatory mechanisms. However, host genomic mechanisms mediating microbial control of intestinal gene expression are poorly understood. Hepatocyte nuclear factor 4 (Hnf4) is the most ancient family of nuclear receptor transcription factors with important roles in human metabolic and inflammatory bowel diseases, but a role in host response to microbes is unknown. Using an unbiased screening strategy, we found that zebrafish Hnf4a specifically binds and activates a microbiota-suppressed intestinal epithelial transcriptional enhancer. Genetic analysis revealed that zebrafish hnf4a activates nearly half of the genes that are suppressed by microbiota, suggesting microbiota negatively regulate Hnf4a. In support, analysis of genomic architecture in mouse intestinal epithelial cells disclosed that microbiota colonization leads to activation or inactivation of hundreds of enhancers along with drastic genome-wide reduction of HNF4A and HNF4G occupancy. Interspecies meta-analysis suggested interactions between HNF4A and microbiota promote gene expression patterns associated with human inflammatory bowel diseases. These results indicate a critical and conserved role for HNF4A in maintaining intestinal homeostasis in response to microbiota.

Dietary palmitic acid induces lipotoxicity in zebrafish via hepatic endoplasmic reticulum stress

Postgraduate student Qianwen Ding[1], **PhD student Zhen Zhang[1]**, **Assistant Research Fellow Chao Ran[1]**, Professor Zhenyu Du[2], **Researcher Zhigang Zhou[1]**
[1]Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Beijing, China, [2]School of Life Sciences, East China Normal University, Shanghai, PR China, Shanghai, China

Palm oil (PO), an alternative lipid source containing high level of palmitic acid (PA, C16:0), has been widely used to replace fish oil in aquafeeds. In this study, we evaluated the potential liver damage induced by PA supplementation in zebrafish. 1-month old zebrafish were fed either low fat diet (LFD), high fat diet (HFD) or HFD comprising different levels of PA (PA4%, PA8%, PA12%) for 4 weeks. The serum ALT(alanine transaminase) and AST (aspartate aminotransferase) were significantly increased in PA 8% and 12% groups compared with the HF group ($P< 0.05$). The expression of UPR markers (Grp78, Chop) was also up-regulated in PA12% group. Further, the responses and potential pathways related to PA hepatotoxicity were analyzed at 24 h post PA treatment in zebrafish liver cells (ZFL). We found PA induced apoptosis in a concentration- and time- dependent manner. The expression of UPR sensors, Grp78 and Grp94, was up-regulated by 50 μM PA ($P<0.01$) and 100 μM PA ($P<0.01$). Blocking experiment indicated that PERK-CHOP and IRE1α-JNK pathways were involved in PA-induced apoptosis in ZFL. Finally, we used zebrafish larvae to verify whether the defined pathways in ZFL were involved in PA lipotoxicity in vivo. In larvae model, the expression of Atg4b, Atg9a and Saa2 were also decreased after blocking PERK-CHOP pathway ($P< 0.01$). Collectively, PA damaged liver of zebrafish via activation of ER stress, which suggested the limitation of high PO inclusion in the aquafeeds for warm water finfish culture. Moreover, the effect of gut microbiota is also being studied in order to explore the anti-PA hepatotoxicity factors. The intestinal microbiotas from the PA8%-fed fish (PA fish), antibiotic (olaquindox)-treated fish or control fish is transferred to germ-free zebrafish fed PA8% diet in which caspase3 and expression of gene markers related to liver damage and ER stress will be evaluated.

Unraveling the role of surface mucus-binding protein and pili in muco-adhesion of Lactococcus lactis

Mickaël Castelain[1]
[1]*INSA de Toulouse, Toulouse, France*

Adhesion of bacteria to mucus may favour their persistence within the gut and their beneficial effects to the host. Interactions between pig gastric mucin (PGM) and a natural isolate of Lactococcus lactis (TIL448) were measured at the single-cell scale and under static conditions, using atomic force microscopy (AFM). In parallel, these interactions were monitored at the bacterial population level and under shear flow. AFM experiments with a L. lactis cell-probe and a PGM-coated surface revealed a high proportion of specific adhesive events (60 %) and a low level of non-adhesive ones (2 %). The strain muco-adhesive properties were confirmed by the weak detachment of bacteria from the PGM-coated surface under shear flow. In AFM, rupture events were detected at short (100-200 nm) and long distances (up to 600-800 nm). AFM measurements on pili and mucus-binding protein defective mutants demonstrated the comparable role played by these two surface proteinaceous components in adhesion to PGM under static conditions. Under shear flow, a more important contribution of the mucus-binding protein than the pili one was observed. Both methods differ by the way of probing the adhesion force, i.e. negative force contact vs. sedimentation and normal-to-substratum retraction vs. tangential detachment conditions, using AFM and flow chamber, respectively. AFM blocking assays with free PGM or O-glycan fractions purified from PGM demonstrated that neutral oligosaccharides played a major role in adhesion of L. lactis TIL448 to PGM. This study dissects L. lactis muco-adhesive phenotype, in relation with the nature of the bacterial surface determinants.

Gene-by-environment interactions drive microbiota variation and response to antibiotics

Dr. Kat Milligan-Myhre[1], Dr. Emily Lescak[1], Ryan Lucas[1], Ruth Isenberg[1]
[1]University of Alaska Anchorage, Anchorage, United States

Background: The role of the host genetic background in the relationship between microbes and host have not been fully explored. Vertebrate animal models used in gnotobiotic host-microbe studies were initially heterogenetic animals that reflected both the genetic and phylogenetic diversity of wild populations. However, recent studies in popular science have focused on highly in-bred models. To better reflect the genetic diversity within vertebrates, we use the evolutionary and biomedical model threespine stickleback fish (Gasterosteus aculeatus) to study the evolution of host-microbe interactions. Environmental and geographic barriers have resulted in hundreds of freshwater stickleback populations in Alaska that originated from the same oceanic ancestors, but evolved in environments that differ in chemical, microbial, and other factors. Thus, they exhibit extensive intra- and inter-population genetic variation that is well characterized, similar to humans. While our initial studies focus on differences in both immune responses to microbes and microbiota community between populations, our long term goal is to examine the role of host genetic background in host-microbe interactions, and the ability of the host to resist changes to the microbiota.
Methods: We have begun to characterize the microbiota of several different freshwater and oceanic populations of stickleback fish. We established a wild-type microbial community in a laboratory setting, and have started laboratory experiments to determine how different populations withstand challenges to their microbiota. To do this, we exposed fish from three different populations to antibiotics and measured differences in development.
Results and conclusions: We successfully established a wild-type-like microbiota in the laboratory. We determined that fish from three different populations exposed to the same antibiotic treatment had different developmental trajectories, indicating that the host genetic background influenced the relationship between the host and the microbiota. Our results suggest that freshwater populations may exhibit more developmental instability than oceanic populations.

The growth promoting effect of dietary nucleotides in fish is associated with an intestinal microbiota-mediated reduction in energy expenditure

Zhigang Zhou[1], Xiaoze Guo[1], Chao Ran[1], Zhen Zhang[1], Suxu He[1], Min Jin[1]
[1]*Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China*

Nucleotides (NTs) have been used as functional nutrients to improve growth and health of animals including fish. In this study, we investigated the bioenergetic mechanism underlying the growth promotion effect of NTs in zebrafish and the associated roles played by the intestinal microbiota. Larval zebrafish were fed control or 0.1% mixed-NTs supplemented diet for 2 weeks. Standard metabolic energy was evaluated by oxygen consumption using a respirometer. The expression of fasting-induced adipose factor (Fiaf), inflammatory cytokines, and genes involved in fatty acid oxidation was tested by qRT-PCR. The intestinal microbiotas from the NTs fed fish or control fish were transferred to 3-dpf germ-free (GF) zebrafish in which oxygen consumption and expression of inflammatory cytokines and fiaf were evaluated. Results showed that NTs supplementation at 0.1% increased the weight gain of zebrafish. The standard metabolic energy was 28% lower in NT fish compared with control. NTs down-regulated the inflammatory tone in the head kidney of fish. Moreover, NTs fed fish had 51% lower intestinal expression of the fiaf gene compared with controls, which accorded with decreased expression of key genes involved in fatty acid oxidation (cpt1a and mcad) in liver and muscle. GF zebrafish colonized with microbiota from fish fed NTs had 25% lower standard metabolism compared with those colonized by control microbiota, while direct NTs feeding of GF zebrafish did not affect standard metabolism, indicating that the reduction in standard metabolism was mediated by the microbiota. Furthermore, GF zebrafish colonized with NT microbiota exhibited down-regulated inflammatory tone and 33% lower fiaf expression compared with control microbiota colonized counterparts. In conclusion, the growth promoting effect of dietary NTs involves two intestinal microbiota-mediated mechanisms that result in reduced standard metabolic energy: (i) lower inflammatory tone; and (ii) reduced fatty acid oxidation associated with increased microbial suppression of intestinal fiaf.

Host-microbe interactions in Atlantic cod larvae

Torunn Forberg[1], Ragnhild Inderberg Vestrum[1], Birgit Luef[1], Ingrid Bakke[1], Yngvar Olsen[2], Per Winge[2], Olav Vadstein[1]
[1]NTNU, Department of Biotechnology and Food Science, Trondheim, Norway, [2]NTNU, Department of Biology, Trondheim, Norway

We have developed a gnotobiotic system for cod larvae, which allows us to investigate host-microbe interactions under controlled microbial conditions. We previously observed that the survival of germ-free cod is consistently higher than for conventionally reared controls. Despite high survival and normal eating behavior, the germ-free cod larvae gain less weight than the conventional. We used a cDNA microarray covering 24000 transcripts to compare the expression in germ-free, conventional and gnotobiotic cod larvae. The gnotobiotic fish were reared in the presence of two cod-derived, non-pathogenic bacteria. At 16 days post hatch, approximately 1500 genes were differentially expressed between germ-free and conventional cod ($p=0.05$, fold change >1.25). The gnotobiotic cod were very similar to the germ-free, with only 22 significantly differently expressed genes. Several transcripts that were moderately upregulated in the germ-free larvae are indicative of fasting; pparα and target genes involved in lipid metabolism, proteolytic enzymes and the appetite reducing peptide YY. In conventional larvae, the microbiota seemed to contribute to higher nutrient availability and digestion; glucose transporters and genes involved in cell division and differentiation were upregulated. While some immune responses were upregulated in conventional cod, others showed higher expression in the germ-free larvae. Genes involved in reactive oxygen formation; myeloperoxidase, cytochrome b-245 and eosinophil peroxidase were down-regulated in both conventional and gnotobiotic fish, contrary to findings in germ-free zebrafish. Atlantic cod are heavily reliant on their innate immune system, and lack cell-surface Toll-like receptors. The immunomodulatory role of bacteria may therefore differ from other fish and mammals.

Zebrafish Olfactory Crypt Neurons have a role in disease resistance against viral pathogens

Jorge Galindo-Villegas[1], Aurora Krause[2], Victoriano Mulero[1], Irene Salinas[2]
[1]Murcia University, Department of Cell Biology and Histology, Faculty of Biology, Institute of Biomedical Research of Murcia-Arrixaca, Espinardo Campus, University of Murcia, 30100, Spain, [2]Center for Evolutionary and Theoretical Immunology, Department of Biology, University of New Mexico, Albuquerque,, USA

Pattern recognition receptors of the immune system are crucial elements in sensing microorganisms. Olfactory sensory neurons are responsible for the detection of chemical stimuli but they are also in direct contact with external microbes. Crypt neurons are unique to teleosts, have enigmatic functions and express only one type of olfactory receptor, the olfactory receptor class A-related 4 (ORA4). We hypothesized that crypt neurons are involved in antiviral immune responses in teleosts. To test it in-vivo, we developed a Gal4 transgenic zebrafish line in which ORA4 can be ablated with the prodrug metronidazole. Our results revealed the timing in which ORA4 cells appear in offsprings and the recovery processes after ablation. But, more importantly ORA4 ablation resulted in increased disease susceptibility to the viral pathogen spring viremia of carp (SVCV). Taken together, our findings highlight a vital role for crypt neurons to not only detect chemical stimuli but also sense environmental danger signals and mount immune responses.

Elucidating the role of microbial communities in cod larval death and survival

Birgit Luef[1], Torunn Forberg[2], Ragnhild I. Vestrum[1], Mari-Ann Østensen[3], Yngvar Olsen[3], Ingrid Bakke[1], Olav Vadstein[1]
[1]NTNU, Department of Biotechnology and Food Science, Trondheim, Norway, [2]Biomar, Trondheim, Norway, [3]NTNU, Department of Biology, Trondheim, Norway

Unfavorable host–microbe interactions are a common phenomenon in aquaculture fish production. Especially the youngest life stages of cultivated fish are prone to negative interactions with opportunistic bacteria, resulting in infections and high mortalities. To establish a more sustainable juvenile production a detailed understanding of the characteristics and the role of the indigenous microflora of fish larvae is essential to improve conditions for the intensive mass rearing of healthy fish.
Due to its complexity, this demands an interdisciplinary approach, combining concepts and methods from microbial ecology, marine larviculture and a variety of state-of-the-art imaging methods. We studied cod larvae reared under three conditions: germ-free, containing a known and defined microbial flora (gnotobiotic) thought to be beneficial for cod larvae survival, and cod larvae grown conventionally with a less controlled microbial community. Within our experiments, there was lower survival of cod larvae grown conventionally compared to germ-free and gnotobiotic reared larvae. Survival was highest for cod larvae grown under gnotobiotic conditions. On day 16 post hatch the dry weight of germ-free grown larvae was significantly lower than for gnotobiotic reared fish larvae, indicating an important role of bacteria in nutrient absorptions.
We utilized correlative light and electron microscopy to characterize in detail the bacterial colonization and also the associated changes in their community structure resulting from stimulation with probiotic bacteria. We obtained integrated information on the scale of one hundred micrometers up to a few nm and thus defined the identity and distribution of organisms, their morphologies, and the relationships between organisms. We characterized bacteria-host interactions, changes in membership ratios and relationships. We hypothesize that the microbial composition in the intestine of cod can increase the host's resistance against pathogenic infection.

Experimental evolution as a tool for understanding and engineering host-associated microorganisms

Cathy Robinson[1,2], Jarrett Lebov[1,3], Elizabeth Miller[1,2], Karen Guillemin[1,2], **Brendan Bohannan[1,3]**
[1]META Center for Systems Biology, University of Oregon, , United States, [2]Institute of Molecular Biology, University of Oregon, , United States, [3]Institute of Ecology and Evolution, University of Oregon, United States

Experimental evolution is a powerful tool for understanding the nature of adaptation, but has rarely been used to understand microbial adaptation to hosts. We used an experimental evolution approach to understand the phenotypic and genotypic determinants of host-association in two different bacterial taxa, Aeromonas sp. strain ZOR001 and Shewanella oneidensis strain MR-1. Shewanella MR-1 is a well-studied environmental microorganism with no known previous association with hosts, but is closely related to a bacterium commonly found in zebrafish intestines. Aeromonas ZOR001 is a member of the zebrafish intestinal microbiota, but has a per capita impact on neutrophil recruitment different from other bacterial isolates from zebrafish. We passaged each of these strains through the zebrafish intestine 20 times. Both strains rapidly increased their fitness in the gut (relative to their ancestor), indicating adaptation. Initial adaptation involved increased rates of colonization from the external environment into the zebrafish, with subsequent adaptation to the gut environment. These results suggest a conceptual model of microbial adaptation to hosts, whereby microbes first adapt to “get in” and then adapt to “get better” in the gut environment. Our results demonstrate that experimental evolution is a powerful tool not only for understanding the nature of host-association, but also for engineering increased host-association by microorganisms.

Fishmeal replacement with insect meal in the supplied diet of three commercially reared fish has differential effect on gut bacterial communities

Efthithimia Antonopoulou[1], Eleni Nikouli[2], Giovanni Piccolo[3], Laura Gasco[4], Stavros Chatzifotis[5], Eleni Mente[2], **Konstantinos Kormas[2]**
[1]Department of Zoology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece, [2]Department of Ichthyology and Aquatic Environment, University of Thessaly, Volos, Greece, [3]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy, [4]Department of Agricultural, Forest and Food Sciences, University of Turin, Turin, Italy, [5] Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Heraklion, Greece

The structure of the gut microbiota is mainly a deterministic feature and is regulated by the available nutrient background and host-related secretions. Commercially reared fish undergo frequent diet substitutions and this renders them an excellent experimental field for disentangling the shaping and responsiveness of gut bacterial communities. In this study, we hypothesized that partial fishmeal to insect meal replacement of the supplied feed in three commercially reared fish species will result in different reshaping of the fish gut microbiota. Intestines of three teleost species Sparus aurata, Dicentrarchus labrax and Oncorynchus mykiss were sampled at the end of different feeding trials with insect meal substitutions (50% for S. aurata and D. labrax, 60% for O. mykiss) and different experimental duration (30, 9 and 10 weeks, respectively) for DNA extraction. The bacterial communities structure was investigated by 16S rRNA gene diversity by 454 pyrosequencing of the V3-V4 region. A total of 598 operational taxonomic units (OTUs) were found in all samples, but in each treatment only 5-20 OTUs dominated (i.e. ≥80% of the cumulative relative abundance), indicating that a few Bacteria thrive in the different gut habitats. Most of the abundant OTUs belonged to the Proteobacteria (Enterobacteriales, Pseudomonadales) Firmicutes (Bacillales, Lactobacillales), Tenericutes, Fusobacteria, Bacteroidetes and Actinoabacteria. Only in the S. aurata and D. labrax gut the dominant OTUs before the substitution were replaced by new dominant ones after the replacement , a clear evidence that the new dietary environment selects for different Bacteria. Additionally, after the insect meal replacement, a higher percentage of new OTUs appeared in the gut microbiota of S. aurata (62.2%) and D. labrax (60.0%) compared to O. mykiss (33.0%) further indicating a lesser community perturbation in O. mykiss. Our results indicate that a substantial insect meal diet replacement in reared fish affects differentially the host organisms gut microbiota.

Fish gut microbiota profiling: Untold stories of technical challenges during sample handling, preprocessing and data analyses

Dr. Karina Gajardo[1], Mr. Alexander Jaramillo-Torres[1], **Dr. Trond M. Kortner[1]**, Prof. Åshild Krogdahl[1]
[1]*Department of Basic Sciences and Aquatic Medicine, Faculty of Veterinary Medicine, Norwegian University of Life Sciences , Oslo, Norway*

Characterization and profiling of the various microbiota populating mucosal surfaces of fish are now receiving high interest, mostly driven by recent advances in and access to high-throughput analytical tools. The gut harbors the largest microbiota population in the body, and it is clear that intestinal microorganisms are essential for the health and normal function of the gut. Based on the understanding that gut dysbiosis seems to be implicated in a number of human gut diseases and disorders, gut microbiota profiling may become a valuable endpoint measurement in order to assess fish intestinal health status and effects of diet, disease, management and environmental factors. However, the current lack of standardized methodology in fish microbial research hampers the development of microbial markers that may be used for diagnostic purposes in fish.

Based on high-throughput 16S rRNA gene amplicon sequencing data from several nutrition studies using Atlantic salmon, we will present an overview of the current understanding on microbial profiles in different compartments in the salmon gut. We will also highlight and discuss important technical challenges related to sampling techniques, sample preprocessing, sequencing and bioinformatic analyses. We believe that these technical challenges are imperative to consider, in order to be able to provide valid descriptions of gut microbiota communities and also meaningful interpretations of host-microbe interactions in the fish gut.

Comparing microbiota between wild and reared fish: exploring microbial clues for aquaculture diversification

Prof. Jaime Romero[1], Mrs Carolina Ramirez[1], Mrs Natalia Catalan[1], Dr. Alejandro Villasante[1]
[1]*Universidad de Chile, Santiago, Chile*

Aquaculture has become one of the fastest growing animal production sectors in the world. In this scenario, Chile has an interesting opportunity for diversification because of its experience in intensive farming (salmonids) and long coastal area, which houses several native species. One of the main challenges for diversification in aquaculture facilities is the control of diseases, particularly during early life stages. Considering the key role of microbiota on healthy status of the host, we use next generation sequencing technology to determine the microbiota composition in fish of commercial interest belonging to the following orders: Pleuronectiformes (flounder, Paralichthys adspersus), Perciformes (yellowtail, Seriola lalandi) and Ophidiiformes (conger, Genypterus chilensis). Knowledge of microbiota may help to improve the cultivation of those species; however, few comparative studies have evaluated the intestinal microbiota composition in farmed versus wild fishes.
The comparison between wild and aquaculture specimens revealed important differences in the composition of the microbiota (ANOSIM and PERMANOVA). Wild flounder showed a predominance of Proteobacteria (70%), while aquaculture flounder were dominated by Firmicutes (60%). Specific taxa were found to be differentially distributed between aquaculture and wild flounder; Bacillus and Pseudomonas were highly represented in aquaculture, meanwhile, Arthrobacter and Psychrobacter were observed in wild flounders. In yellowtail, a similar tendency in phylum distribution was observed, but a total of thirteen genera were differentially represented between the two origins. In conger, Firmicutes were dominant in reared fish, but Tenericutes were dominant in wild fish.
These results are interesting in the context of co-evolution between the host and its microbiota because co-evolution is believed to have been an important mechanism in the formation of the host-gut microbe relationship. However, factors such as diet may influence the composition of the gut microbiota and consequently the metabolic functions of intestinal microbes, with effects on the host's nutrition and immune defense.

Fish microbiota ontogeny: usefulness for aquaculture and natural population conservation

Nicolas Derome[1]
[1]*Universite´ Laval, Quebec, Canada*

Fish associated microbial communities (e.g. gut microbiota) play multiple functions that are indispensable for their host biology: nutrient metabolization, protection against pathogens, synthesis of essential amino acids, vitamins, hormones, etc.
Microbiota taxonomic and functional compositions are highly dynamic and vary strongly according to successive host organism developmental stages (i.e. host ontogeny). Also, microbiota composition is controlled by main two factors: environment and host genotype. During the earlier developmental stages, fish larvae will recruit pioneering symbionts from their proximal environment. Therefore, environmental community composition is a key factor for sequential recruitment of beneficial microbial functions, promoting both nutrition efficiency and resistance against opportunistic pathogens, for instance. However, in aquaculture settings, fish are usually exposed to very controlled microbial environments, which are different from the wild, thus potentially compromising recruitment of key symbionts.
In this talk, we will state on the current knowledge on gut microbiota ontogeny in teleost fishes, document the respective influence of factors that are mainly involved in shaping microbiota composition, and discuss their importance regarding the development of alternative aquaculture practices promoting recruitment of more resilient microbiota, both in the context of wild population stocking and food production.

Influence of environmental factors on the microbiota associated with developing cod larvae (Gadus morhua)

Ingrid Bakke[1], Kari Attramadal[1], Olav Vadstein[1]
[1]NTNU, Norway

After developing inside the egg in a bacteria-free environment, upon hatching fish larvae are exposed to high numbers of bacteria in the ambient water. The microbial conditions in aquaculture systems differ from those in the natural environments. Due to density dependent feeding and excretion (in limited tank volumes), there is a high potential for growth of opportunistic bacteria in rearing systems. Furthermore, the diet used in aquaculture systems may select for other members in the gut microbiota than under natural conditions. For marine fish larvae, negative bacteria-larva interactions are known to cause problems with low and unpredictable viability. As part of the EU project "Promicrobe: Microbes as positive actors for more sustainable aquaculture" (2009-2013), we designed experiments to investigate the influence of live feed and water on cod larval microbiota. We also examined the potential for manipulating the larval microbiota by the introduction of bacterial strains through live feed and rearing water. In this presentation, we summarize the results. The most important conclusions were: a) different live feed diets had a minor impact on the larval microbiota, b) different rearing water significantly affected the larval microbiota, c) introduced strains, originating from the gut of cod larvae were only transiently observed as part of the larval microbiota, and d) the composition and diversity of the larval microbiota changed considerably during larval development.

SalmoSim: Exploring the microbial basis of Atlantic Salmon energetics via a synthetic intestinal system

Martin Llewellyn[1]
[1]*University of Glasgow, Scotland, UK*

Salmon energetic phenotypes are composites of several interlinked traits: metabolic rate, body fat content, growth, energy harvest from food, energy economy in times of starvation. These traits underpin concerns around salmon nutrition. Significant energetic variation exists in both wild and farmed salmon with multiple possible drivers – both genetic and environmental. Importantly a wealth of new data indicates a role for intestinal microbiota – the bacteria that live in the guts of all vertebrates - in determining host energy metabolism. Via the transfer and maintenance of gut bacteria from metabolically different fish from farmed and wild settings into a gut model we aim to establish how bacterial fermentation underpins differences in energy harvest from feed. Once these 'artificial' bacterial communities are established, a final exploratory phase of the project will involve their transplantation into laboratory reared juvenile salmon to evaluate their potential impact on host metabolism.

Catastrophe and fast dispersal dynamics of a gut bacterial community following sub-lethal antibiotics

Brandon Schlomann[1], Travis Wiles[1], Karen Guillemin[1], Raghuveer Parthasarathy[1]
[1]*University of Oregon, Eugene, United States*

Antibiotics can cause large crashes in gut bacterial diversity at time scales shorter than a day, though the recovery and reassembly of the communities can take months. The fate of the survivors of this process - bacteria that encountered antibiotics but remain viable - is a mystery. I'll present recent work that aims to address this by using intentionally sub-lethal doses of antibiotics to perturb well-defined microbial communities in a model organism, the zebrafish. Light Sheet Fluorescence Microscopy is used to image a commensal species of Vibrio responding to antibiotic perturbations in the guts of live, larval fish. We find that sub-lethal concentrations of different classes of antibiotics induce similar physical responses in Vibrio, namely filamentation and reduction of motility. The arrested bacteria then aggregate and can be ejected via peristalsis, resulting in large population collapses that disperse bacteria in to the environment, potentially impacting host-host transmission. These observations suggest that even if bacteria populations survive antibiotic exposure, they may still contribute to loss of diversity in the gut, and also may impact the greater metapopulation structure.

Tributyltin exposure alters post-embryonic growth and intestinal microbiota assembly in zebrafish

Doctor Sol Gomez de la Torre Canny[1], Doctor Olaf Mueller[1], Doctor John F. Rawls[1]
[1]Department of Molecular Genetics and Microbiology, Duke University, Durham, United States

Obesity is a ubiquitous public health problem that warrants the study of environmental factors that, by regulating energy balance, promote the storage of excess energy in adipose tissue. Two important environmental factors that promote adipose tissue size are microbial communities residing in the intestine, and environmental chemicals that may act as obesogens. We developed an in vivo model to test the obesogenic effect of chronic tributyltin (TBT) exposure in post-embryonic zebrafish and to study the effect that this chemical has on intestinal microbiota. Zebrafish develop multiple adipose tissue depots, which consist of white adipocytes that display morphological and molecular homologies with those of mammals. Using fluorescent lipophilic dyes to measure adipose tissue in vivo and gnobiotic husbandry methods we found that microbiota colonization, as in mammals, promotes adipose tissue accumulation in zebrafish. Also, we show that 21-day exposure to TBT at a nominal dose of 1 ug/l was sufficient to increase the growth of adipose tissue in zebrafish compared to control animals. Unexpectedly, TBT exposure also inhibited somatic growth as early as 14 days post-exposure and at a dose as low as 0.1 ug/l. High-throughput sequencing of 16S rDNA revealed specific effects of TBT on intestinal bacterial communities by 14 days post-exposure such as an increased Chitinibacter spp. abundance. Together, our results indicate that TBT exposure promotes adipose tissue deposition even in the absence of a high fat diet, and affects other distinct aspects of post-embryonic growth and intestinal microbiota assembly. This work provides the first comprehensive analysis of the effect of a chemical obesogen on zebrafish somatic growth, adipose tissue accumulation, and intestinal microbiota composition, providing a critical frame of reference for future studies to test for mechanistic interactions between environmental toxins, microbiota, and animal hosts.

Inter-host dispersal is sufficient to overwhelm host factors in the assembly of the zebrafish gut microbiome

Dr. Adam Burns[1], Dr. Elizabeth Miller[1], Meghna Agarwal[2], Dr. Annah Rolig[2], Prof. Kathryn Milligan-Myhre[3], Dr. Steve Seredick[4], Prof. Karen Guillemin[2], Prof. Brendan Bohannan[1]
[1]University of Oregon, Institute of Ecology and Evolution, Eugene, United States, [2]University of Oregon, Institute of Molecular Biology, Eugene, United States, [3]University of Alaska Anchorage, Anchorage, United States, [4]University of Oregon, Institute of Neuroscience, Eugene, United States

Understanding how the diverse collections of microorganisms associated with fish and other animals, collectively referred to as their "microbiome," contribute to host health requires understanding the mechanisms driving microbiome composition. However, it has proven difficult to identify consistent host factors that explain variation in microbiomes across hosts, despite large-scale sampling efforts. While ecological theory predicts that the movement, or dispersal, of individuals can have profound and predictable consequences on community assembly, its role in the assembly of animal associated microbiomes remains unknown. Here we show how inter-host dispersal contributes substantially to microbiome variation, overwhelming the effects of individual host factors, and its effects are consistent with ecological theory. We manipulated dispersal among wild-type and immune-deficient myd88 knock-out zebrafish and observed that inter-host dispersal had a large effect on the diversity and composition of intestinal microbiomes. Inter-host dispersal was strong enough to overwhelm the effects of host factors, largely eliminating differences between wild-type and immune-deficient hosts, regardless of whether dispersal occurred within or between genotypes, suggesting dispersal independently alters the ecology of the microbiome. Our observations are consistent with a predictive model that assumes metacommunity dynamics, and are likely mediated by dispersal-related microbial traits. These results demonstrate the importance of microbial dispersal to animal microbiomes and motivate its integration into the general study of host-microbe systems.

Differences in gut microbiota of distinct strains within two species of ictalurid catfish are not explained by estimated genetic distances among hosts'

Jacob W. Bledsoe[1], Geoff C. Waldbieser[2], Kelly S. Swanson[3], Brian C. Peterson[4], Brain C. Small[1]
[1]Aquaculture Research Institute, University of Idaho , Hagerman, United States, [2]Warmwater Aquaculture Research Unit, USDA-ARS, Stoneville, United States, [3]Department of Animal Science, University of Illinois at Urbana-Champaign, Urbana, United States, [4]National Cold Water Marine Aquaculture Center, USDA-ARS, Franklin, United States

Channel catfish Ictalurus punctatus represent the greatest market share of U.S. aquaculture production, both in terms of total economic value and numbers produced. The blue catfish I. furcatus is also of great interest to U.S. aquaculture, because interspecific crosses (I. punctatus x I. furcatus) produce offspring with hybrid vigor and desirable phenotypes such as increased disease resistance and fillet yield. Ictalurid catfish have received much breeding attention, and multiple aquaculture strains with unique performance traits exist within both species, yet little is known about their intestinal microbiota. As such, we aimed to characterize and compare the gut microbiota of six strains of ictalurids (3 I. punctatus & 3 I. furcatus, n = 10 each) (nested design, strain within species) using 16S rRNA V4 sequencing to determine if gut microbiota are correlated with host genetics of strains within the two species. Fish were reared to 193 dph in indoor flow-through tanks, receiving the same diets up to sampling. In addition, host genetic data were gathered from separate individuals from each strain (n=20) in the form of 22 microsatellite loci. Results indicate that microbial alpha-diversity in the three strains of I. punctatus is greater than that of the I. furcatus strains. Differential abundance analysis of OTUs detected within strains of each species, using DESeq2, identified differences in important microbial taxa including: Aeromonadaceae, Enterobacteriaceae, and Pseudomonas. Despite those differences, no shifts in overall beta diversity between strains within species were detected by PERMANOVA and microbial beta-diversity metrics showed no correlations with host genetic distances (Slatkin's-Rst) according to Mantel tests. While microbiota and genetic distances were not correlated, differences in alpha-diversity and differentially abundant OTUs among species reared in the same environment indicate that host-genotype does have an influence on the gut microbiota assemblages in fish.

Water temperature and feeding live yeast impacts growth and gut microbiota of rainbow trout

David Huyben[1], Li Sun[1], Anders Kiessling[1], Johan Dicksved[1], Torbjörn Lundh[1]
[1]*Swedish University of Agriculture (SLU), Uppsala, Sweden*

Heat-extruded diets of inactivated yeast have been shown to replace 40% of fish meal without negative effects on fish growth and gut microbiota, although impacts such as water temperature and high amounts of live yeast are relatively unknown. Rainbow trout (129g) were fed either a cold-pelleted fishmeal or yeast-based diet (40% replacement; 8 log CFU of Saccharomyces cerevisiae) and reared in water temperatures of either 11C or 18C for 6 weeks. Afterwards, content and mucosa scrapings were collected from the distal intestine of three fish per tank (n=12). For yeast, samples were agar plated, extracted in formic acid and identified using MALDI-TOF. For bacteria, samples were extracted for DNA, PCR amplified and barcoded using Illumina primers. Pooled samples were sequenced using Illumina MiSeq and data were analysed using QIIME and R software. Significant effects on specific growth rate and weight gain were found, which were lowest for fish fed the yeast-coldwater diet, whereas fish fed the fishmeal-coldwater diet had a significantly higher thermal growth coefficient than the warmwater fish. Intestinal content and mucosa of fish fed the yeast diets contained 7 log CFU of yeast compared to 2 log CFU in fish fed the fishmeal diets, respectively. Blood and intestinal pH were both higher for fish fed the fishmeal-coldwater diet than the warmwater diets. Both yeast and fishmeal diets were composed of Lactobacillales and Vibrionales. Intestinal content and mucosa mainly contained Mycoplasmatales that were more abundant in warmwater fish whereas Lactobacillales were less abundant. Insignificant fish growth and lower abundance of beneficial bacteria in the gut suggested that rearing rainbow trout at 11C is more optimal than 18C and feeding cold-pelleted diets of live yeast.

Effects of the probiotic Phaeobacter inhibens against fish pathogens in non-axenic algae and copepod systems

Bastian Barker Rasmussen[1], Katrine Ege Erner[1], Dr. Mikkel Bentzon-Tilia[1], Prof. Lone Gram[1]
[1]*Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs. Lyngby, Denmark*

Several commercially important marine finfish require live feed at the larval stage. Live feed can act as a potential entry point for pathogens such as bacteria of the family Vibrionaceae. Antibiotics are still used to control these pathogens and sustainable alternatives are sought after. Tropodithietic acid (TDA) producing Roseobacter group bacteria have probiotic potential as they are able to protect live feed e.g. rotifers and Artemia as well as larvae of turbot and cod against pathogenic vibrios. Advances in the breeding of copepods for live feed have made them relevant in aquaculture. The purpose of this study was to investigate if TDA-producing Phaeobacter inhibens could inhibit Vibrio anguillarum in non-axenic algae and copepod systems. Preliminary data showed that GFP-tagged V. anguillarum colonized the outer surface and gut of the copepods indicating that they could act as potential vectors for the pathogen. P. inhibens was able to inhibit the growth of V. anguillarum in non-axenic cultures of the copepod feed Rhodomonas salina. However, V. anguillarum did, unexpectedly, not grow in copepod cultures as it did in other live feed systems. On-going experiments are addressing this issue, setting up a qPCR protocol for easy quantification of the pathogen and testing the potential of P. inhibens as an inhibitor of V. anguillarum in copepod cultures.

Description of microbiota of Atlantic bonito larvae (Sarda sarda), isolation of putative probiotics and use for the rearing of sea bass larvae (Dicentrarchus labrax)

Dr Pavlos Makridis[1], Dr Elena Sarropoulou[2], Dr Nikos Papandroulakis[2], Dr Pascal Divanach[2]
[1]Department of Biology, University of Patras, Patras, Greece, [2]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Center for Marine Research, Herakleion, Greece

Bonito larvae (Sarda sarda) were reared in a mesocosmos system until 21 days after hatching. The total number of aerobic heterotrophic culturable microbiota was determined by culture on Marine Agar and the presumptive number of Vibrios by culture of larval homogenates on TCBS agar. Five larvae were individually homogenized and plated in solid media 0, 7, 14 and 21 days after hatching. Representative bacterial strains were isolated and identified by 16S rDNA sequencing. A phylogenetic tree of the isolated and characterized species was constructed.
Phaeobacter sp. was isolated from yolk-sac larvae and was pure cultured and stored at -80oC. It was used in in vitro tests to determine its ability to inhibit growth of bacteria fish pathogens and thereafter it was in a large scale trials with rearing of seabass larvae. The microbiota of seabass larvae was characterized by classical microbiological techniques as well as by DGGE analysis.
Survival 60 days after hatching was significantly higher ($P<0.05$) in tanks added probiotics, whereas there were no differences in terms of growth between the two treatments. Total numbers of bacteria was similar in the two groups, but presumptive Vibrios were significantly lower in treatment added probiotics. DGGE analysis showed that microbial diversity in general was highest 4 days after hatching and it gradually decreased. Microbial diversity was lower in the fish added probiotic compared with the control treatment.

Steering the gut microbiome of tilapia larvae with microbial diets

Dr marc Verdegem[1], Dr christos giatsis[2], MSc Joost van Loo[1], MSc Bram Rohaan[3], Dr. Johan Verreth[1], Dr. Hauke Smidt[1], Dr Javier Ramiro-Garcia[1], Dr Detmer Sipkema[1]
[1]wageningen university, Wageningen, Netherlands, [2]Evonic, Germany, [3]Yellow Tail Farm, Netherlands

Fish live and share their environment with microorganisms. The autochthonous microbiota of fish mostly originates from those present in their feed and water. However, the fish gut seems to be selective for the establishment of certain species, while at the same time being hostile to others. Despite the significant effort in defining the forces that impact the assembly of gut microbial communities, the underlying mechanisms attributed to the impact of the environment over the host selectivity are still poorly understood. In the present study the role of feed microbiota on gut communities of tilapia larvae was investigated. Fish were fed three experimental diets incorporated with sludge produced under aerobic, methanogenic or denitrifying conditions. We performed 16S rRNA gene sequence-based comparisons between gut microbial communities from different treatments and we associated them with the ones present in feed and water. Gut microbiota shared a much higher number of OTUs with microbiota in sludge-based feeds than with water, resulting in distinct gut communities between treatments. This finding implies that the tilapia gut microbiota has a certain plasticity, which makes it amenable to interventions. Nevertheless, in spite of observed changes in microbiota composition between treatments, a persistent core gut OTUs was maintained in all treatments.

Managing the microbiota in larval rearing: On the use of K-selection as a microbial community management strategy

Olav Vadstein[1], Kari Attramadal[1], Ingrid Bakke[1], Yngvar Olsen[2]
[1]NTNU, Department of Biotechnology and Food Science, Trondheim, Norway, [2]NTNU, Department of Biology, Trondheim, Norway

It is now well documented that the microbial conditions in aquaculture systems is a major reason for poor viability of many species, and it is caused by detrimental host-microbe interactions. However, usually no known pathogens are detected. Thus, the problem seems more to be rooted in opportunistic bacteria invading hosts that are susceptible due to e.g. stress and immunosuppression. High frequency of opportunist in aquaculture systems can be predicted from the occurrence of frequent disturbances (disinfection, feed addition), which should select for opportunists – or in ecological terms, r-strategists. The most common solution for solving negative microbial effects is the use of probiotics. However, even though addition of probiotics will change the composition of the microbiota, this approach will not do anything with the reason for the problem and it may create ecological instability. The ecological counterpart to r-strategists is K-strategists. They are selected for in crowded environments with high competition – an unfavourable environment for r-strategic opportunist. This talk will present the background for the ecological analysis given above, give examples on how to obtain K-selection in an aquaculture system, and also give some examples on the beneficial effect of K-selection on larval viability.

Microbial control of rearing water

Dr Kari Attramadal[1], Dr Ingrid Bakke[1], Dr Olav Vadstein[1]
[1]NTNU, Trondheim, Norway

A common challenge in land based aquaculture systems, and shown across species, is losses due to unfavourable conditions and disease outbreaks linked to opportunistic bacteria. A popular approach to prevent this is to attempt to reduce the load of bacteria in the systems by disinfection. This is however not possible or sufficient in the majority of systems, because disinfection has a non-lasting effect on the numbers and a destabilising effect on the composition of bacteria. In most systems, the water exchange rates and organic loading applied for biological reasons allow for microbial regrowth in the rearing tanks. The result of uncontrolled regrowth is selection for opportunistic bacteria and large variations in the microbial composition of "replicate" rearing tanks, which reduces reproducibility of the production. Hence, methods to increase reproducibility and to reduce the chances of disease outbreaks are needed. In several experiments we have studied how it is possible to influence the quantitative and qualitative bacterial environment of marine larvae. Selection pressure can be used to establish and maintain stable and beneficial microbial communities in the rearing water. Water treatment technology that promotes K-selection (which disfavour the opportunists) has shown very promising results for several marine species. By providing an environment dominated by friendly bacteria for the fish, and excluding pathogens, the chance of developing healthy host-microbe relationships and produce healthy juveniles is increased. If operated in the right way, recirculating aquaculture systems (RAS) promote K-selection because of the long hydraulic retention time of the water in the system, the large area available for biofilm and the stable microbial carrying capacity throughout the system. In the ongoing MicStaTech project, a simple model is being developed to describe the competition between r- and K-strategist bacteria in a larval rearing tank depending on system type, flow, disinfection and feeding/removal of organic matter.

Risk and benefit evaluation: Low dose antibiotics exposure and the gut health of fish

Professor Meiling Zhang[1], Li Zhou[1], Professor Zhen-Yu Du
[1]*East China Normal University, Shanghai, China*

As the antibiotics pollution in water, aquatic organisms face long-term risk of exposure to antibiotics in water environment. However, the influence of antibiotics exposure on the gut health of fish has not been sufficiently considered, especially for the antibiotics residues in water. Sulfamethoxazole (SMZ) and tetracycline (OTC) are two main antibiotics residues in water environment in China and the influence of these antibiotics exposure on the gut health of zebrafish were studied by evaluating the microbiota composition, intestinal histology, enzyme activity, intestinal antioxidant capacity and innate immune activity. The results indicated that low dose antibiotics exposure increased the intestinal digestive enzymes which increase the weight gain of fish, but it also decrease the survival rate of fish after the Aeromonas hydrophila challenge. Antibiotics exposure destroyed the gut health by altering the structure of the gut microbiota, decreasing the goblet cells, flattening the villi and declining alkline phosphatase(AKP), acid phosphatase(ACP) activities in gut. Our study suggested that when we control the bacteria in aquaculture, we need to systematically evaluate the risk of the strategy.

Rearing water treatment influences the microbiota and transcript profiles of cod larvae.

MSc Ragnhild Inderberg Vestrum[1], PhD Kari Johanne Kihle Attramadal[1], PhD Per Winge[2], PhD Keshuai Li[2], PhD Yngvar Olsen[2], Dr.Scient Atle M. Bones[2], Dr.Philos Olav Vadstein[1], PhD Ingrid Bakke[1]
[1]NTNU, Department of Biotechnology and Food Science, Trondheim, Norway, [2]NTNU, Department of Biology, Trondheim, Norway

We have previously shown that microbial stability in the rearing water increases survival and growth of cod larvae, and that recirculating aquaculture systems (RAS) are compatible with microbial stability. Here, our aim was to assess how water treatment influences the larval microbiota and to examine potential differential host responses at the gene expression level.

Cod larvae were reared with two different rearing water systems: RAS and a flow-through system (FTS). The water microbiota was examined using 16S rDNA PCR/DGGE strategy. RNA extracted from larvae at 8, 13, and 17 days post hatching was used for microarray gene expression and microbiota analysis. cDNA was synthesized and used for 16S rRNA amplicon pyrosequencing of larval microbiota.

Both water and larval microbiota differed significantly between the systems. The most profound difference in larval microbiota was a high abundance of Arcobacter (Epsilonproteobacteria) in FTS larvae (34% ±9). Arcobacter includes several species that are known pathogens for humans and animals. In total 4 phyla were identified for all larvae: Actinobacteria, Bactereoidetes, Firmicutes, and Proteobacteria.

Larval responses at transcriptional level were investigated using an oligonucleotide gene expression microarray covering approximately 24 000 genes. Interestingly, the transcripts that were most upregulated in the FTS compared to RAS larvae generally appeared to be associated with host responses to pathogens and infections.

In conclusion, different water treatment systems induced differences in the larval microbiota. FTS larvae showed up-regulation of several transcripts that could be associated with responses to microbial stress, consistent with the hypothesis that RAS promotes microbial stability.

Control of virulent Aeromonas hydrophila in warm water fish species

Professor Mark Liles[1], Charles Thurlow[1], Dr. Mohammad Hossain[1], Dr. Dawei Sun[1], Associate Professor Joseph Newton[1], Associate Professor Jeffery Terhune[1]
[1]Auburn University, Auburn, USA

A highly clonal population of virulent Aeromonas hydrophila (vAh) has caused the loss of over 10 million kilograms of channel catfish across the southeastern United States since 2009. Phylogenomic and epidemiologic analyses suggest that U.S. catfish isolates emerged from the importation of Asian carp, with outbreaks of A. hydrophila in carp species documented in China since 1989. We have developed two strategies for vAh control, using either probiotic bacteria to prevent disease or a live, attenuated vaccine. Probiotic studies: We have identified Bacillus spp. strains that have the ability to reduce mortality due to multiple bacterial pathogens, including vAh and Edwardsiella ictaluri. The effect of probiotic-amended feed (10^6 CFU spores/g feed) over 10 weeks on catfish growth performance indicated that B. velezensis AP193 induced a mean 14% increase in growth compared to control fish and when challenged with E. ictaluri the fish fed strain AP193 had the best survival rates compared to control fish. In a 10 week pond trial (n=4 probiotic or control ponds) strain AP193-fed fish showed a 9% increase in growth relative to control fish and a 53% reduction in total phosphorus in pond water was observed relative to control ponds. These data suggest that B. velezensis AP193 has the ability to promote catfish growth and improve pond water quality. Vaccine studies: Comparative genomics of vAh strains from the US and China led to the identification of many genetic loci that are uniquely present in vAh strains, including a novel O-antigen biosynthesis gene cluster. Genetic knockouts in the ymcABCD operon were found to attenuate vAh virulence and these mutations could be complemented through the use of a vector containing the complete ymcABCD operon. Furthermore, the ymcA mutant induced an adaptive immune response and protected catfish from challenge with wild-type vAh in aquaria and pond studies.

Effects of probiotic yeasts in the microbiota composition of zebrafish larvae (Danio rerio)

Dr. Mario Caruffo[1], **Dr. Paola Navarrete[1]**
[1]INTA-University of Chile, Santiago, Chile

Yeasts are part of the microbiota of healthy fish gut. We previously identified different yeast species from the gut of wild and reared fish, like Cillus gilberti, Seriola lalandi, and salmonids Salmo salar, Oncorhynchus kisutch and O. mykiss. Some yeast strains showed protective effects against Vibrio anguillarum in zebrafish larvae, modulating the immune response and pathogen load in infected larvae. In this study, we wanted to explore if protective yeasts are able to modulate the bacterial microbiota composition and whether this bacterial modulation and larval protection requires viable yeasts or just the presence of Microbe-Associated Molecular Patterns (MAMPs) elements. We tested the effects of viable and heat-inactivated yeasts in total bacterial concentration and the relative abundance of bacterial taxa of the microbiota. Yeasts were administered through immersion at a concentration of 10^7 CFU/mL in 4 dpf zebrafish larvae. We included a commercial probiotic yeast (Saccharomyces boulardii CN CM I745, Perenteryl®, Merck) to test its performance in this fish model. The results showed that the commercial yeast is able to colonize the larvae as the protective yeasts. Also, total bacteria were not affected by the viable or heat-inactivated yeasts, while the relative abundance of cultured bacteria was similarly affected by the addition of viable or heat-treated yeasts. We are currently working on the identification of the bacterial taxa and determining if yeast viability is necessary for the protection against the V. anguillarum infection. These results will give more details about the mechanisms involved in the probiotic effects of yeasts.

Advances in developing zebrafish as a long-term gnotobiotic model

Ellie Melancon[1,2], Sophie Sichel[1,3], Dr Karen Guillemin[1,3], Dr Judith Eisen[1,2]
[1]Microbial Ecology and Theory of Animals Center for Systems Biology, University of Oregon, Eugene, United States, [2]Institute of Neuorscience, University of Oregon, Eugene, United States, [3]Institute of Molecular Biology, University of Oregon, Eugene, United States

Zebrafish are an emerging model for investigating the mechanisms underlying host-microbe interactions. The vast majority of gnotobiotic zebrafish research to date has focused on larval stages, in part due to the challenges of long-term germ free (GF) zebrafish husbandry. Understanding the myriad ways resident microbes influence host development and physiology requires rearing zebrafish GF beyond early larval stages, with the ultimate goal of rearing them GF throughout the lifespan. The challenges of accomplishing this goal mirror those of running a miniature, specialized zebrafish facility. Developing standardized husbandry conditions appropriate for rearing zebrafish GF throughout their lifespan requires consideration of animal housing, water quality, waste removal, nutrition, labor, and expenses. We present our advances in developing axenic feeding practices and continuous flow cultures that extend our ability to rear zebrafish GF beyond larval stages. The innovative approaches we are developing should allow zebrafish to become a powerful gnotobiotic vertebrate model in which large-scale, long-term husbandry is routinely possible.