



The potential of functional feeds to improve fish welfare

EATiP Forum on Innovations to support Fish Welfare (26 November)

Charles McGurk, Global Health R&D Manager, Skretting Aquaculture Research Centre



Our mission
*feeding
the future*

30
PRODUCTION
PLANTS IN 18
COUNTRIES



>2.3M
TONNES OF
FEED



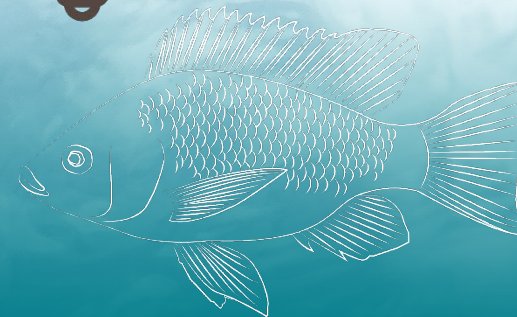
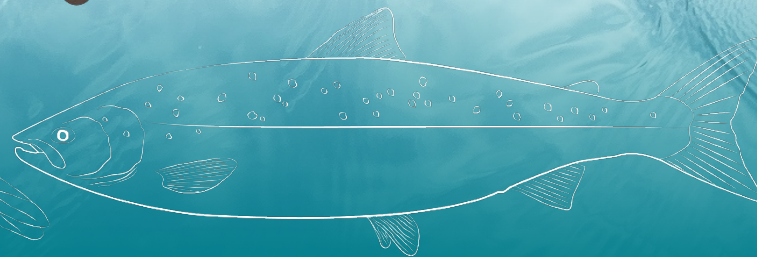
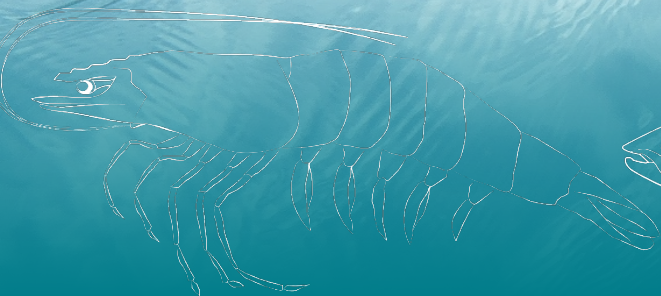
>3,500
EMPLOYEES



11
RESEARCH
& VALIDATION
STATIONS IN 9
COUNTRIES



€15M
ANNUAL
INVESTMENT
IN R&D



Skretting Aquaculture Research Centre

Established
1989

Collaborations with
>60
research
organisations

>140

Employees

>25

Nationalities

40

Researchers



>30
PhDs

Core competencies

Nutrition

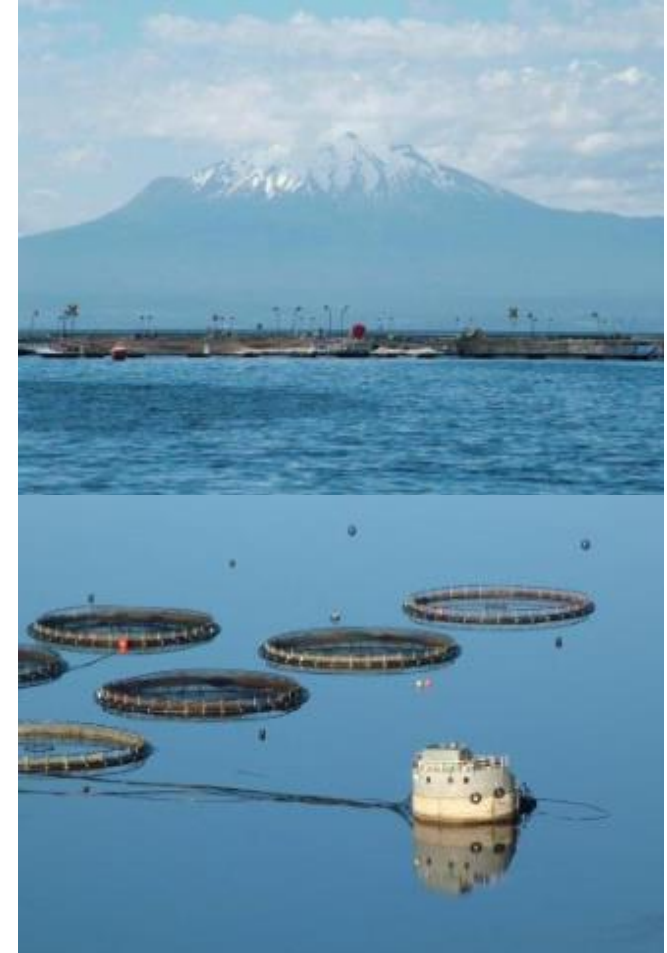
Feed Production

Health

Research on
9 key species



Disease is a major threat to the future of aquaculture

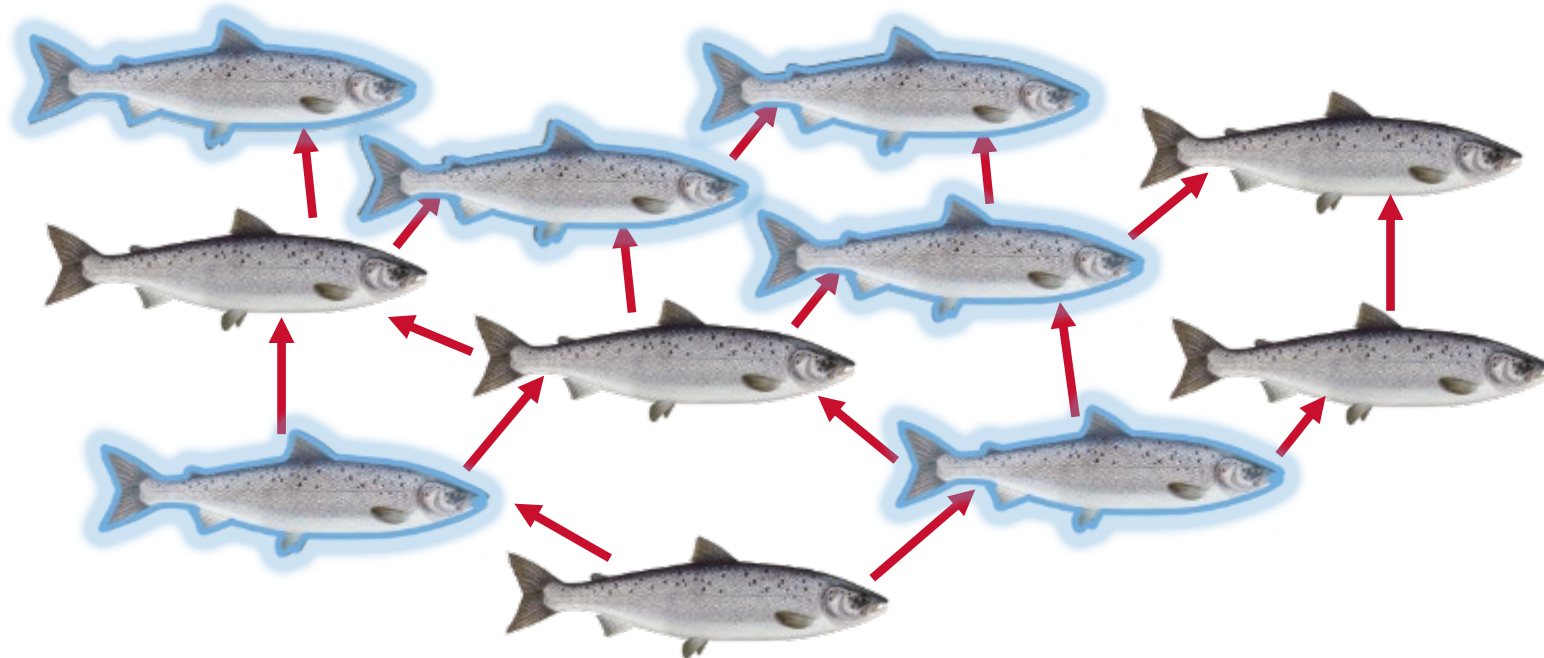


Focus on prevention

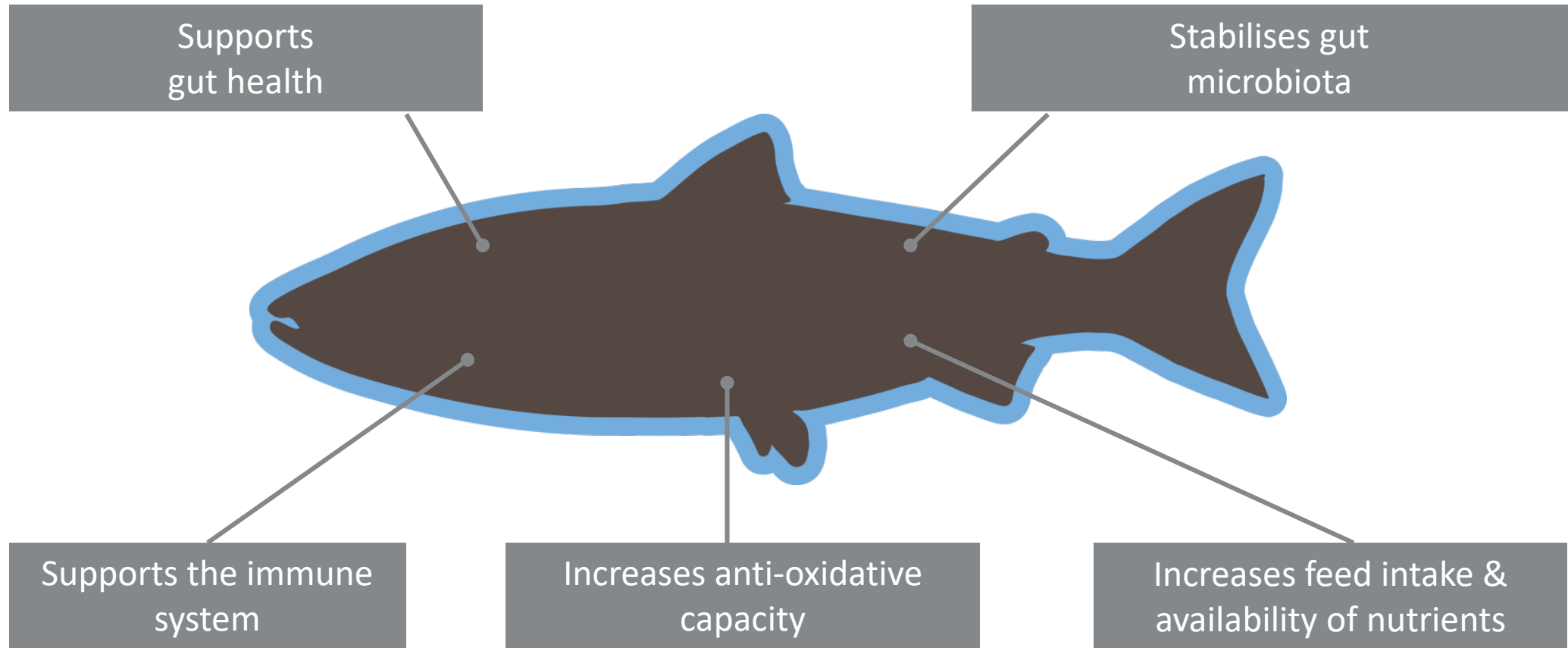
- Move from a therapeutic to a prophylactic approach: focus on prevention
- Holistic approach:
 - Epidemiology
 - Biosecurity
 - Genetic selection
 - Vaccines
 - **Health / Functional diets**
 - Integrated Pest Management

Functional nutrition: potential impact on 'herd immunity'

- Strengthen inherent defences
- Reduce pathogen replication
- Limit pathogen excretion and subsequent challenge pressure



Functional nutrition: principal modes of action



Combating sea lice through functional nutrition

Aquaculture Nutrition

Aquaculture Nutrition 2014

doi:10.1111/an.12222

Reducing sea lice (*Lepeophtheirus salmonis*) infestation of farmed Atlantic salmon (*Salmo salar* L.) through functional feeds

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Abstract
Health diets for Atlantic salmon have become an important component of the integrated pest management strategies targeting sea lice. A challenge trial was performed to examine the effect of supplementing salmon diets with other immunostimulants or essential oils. One control and four experimental diets containing immunostimulants or natural essential extracts were fed to Atlantic salmon in triplicate tanks for 4 weeks before challenging the fish with the sea lice copepods. Prevalence of infection was 300%, and the mean abundance of infection was 212. The lowest mean for count of 17 per fish (P < 0.05) was found in the group fed a mix of natural identical plant extracts (PN 6). This represents a 20% reduction in infection, showing the potential for health diets to be employed as a tool to help control sea lice. To gain an understanding of the mechanisms of action involving this protection, fish fed the control diet and fish fed the PN 6 diet were compared using quantitative biology of the epidermis and proteomic analysis of epidermal mucus. No significant differences were seen in the thickness of the epidermis or mucus cell percentage area, but differences in expression were seen for a number of proteins, including heat shock proteins, in epidermal mucus.

Key words: Atlantic salmon, health diets, histology, infection, proteomics, sea lice

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Journal of Fish Diseases

doi:10.1111/jfd.12214

Investigating the underlying mechanisms of temperature-related skin diseases in Atlantic salmon, *Salmo salar* L., as measured by quantitative histology, skin transcriptomics and composition

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¹Stavanger Aquaculture Research Centre, Stavanger, Norway; ²National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway; ³Institute of Aquaculture, University of Stirling, Stirling, UK

Abstract
Skin integrity is recognized as of vital consideration for both animal welfare and final product quality of farmed fish. This study examines the effects of three different rearing temperatures (4, 10 and 16 °C) on the skin of healthy Atlantic salmon post-smolts. Changes in skin condition were assessed by the means of skin composition analysis, quantitative histology assessments and transcriptome analysis. Level of protein, vitamin C and vitamin E was significantly higher at 16 °C compared with 4 °C. Quantitative histology measurements showed that the epidermal thickness decreased from low to high temperature, whereas the epidermal area comprising mucus cells increased. The difference was only significant between 4 and 16 °C. Both high and low temperature exhibited significant change in the skin transcriptome. A number of immune-related transcripts responded to both temperatures. Contrary to well-described immunosuppressive effects of low water temperature on immune response, a subtle increase in skin-mediated immunity was observed, suggesting a pre-activation of the innate immune system at 4 °C. Upregulation of a number of heat-shock proteins correlated with a decrease in epidermal thickness suggested a stress response in the skin at high temperature. The results demonstrate distinctive temperature-related effects on the skin of Atlantic salmon.

Keywords: *Salmo salar*, skin histology, skin composition, immune immunity

Introduction
Salmon farming is among the world's fastest growing food producing industries. Expanding the industry into new areas outside of the salmon's natural distribution opens questions about the impact of temperature on the fish. There has been a noted concern about how climate change, particularly the impact of global warming, will affect salmonids (Dempsey, O'Connell & Colbourne 2003; Jensen & Jensen 2009). It has been shown that one low chronic stress on salmonids is the disease resistance in fish (Pickering & Pottinger 1998), and it is also well known that drastically stressed fish are more susceptible to infections with pathogens like sea lice (MacKenzie 1999). Some pathogens affecting fish are temperature-modulated, with pathogenicity related to a specific water temperature range. At low temperatures, salmon are more susceptible to skin lesions, including winter ulcers, associated with *Aeromonas* infection (Brenneis, Hjeltnes & Hjeltnes 1978; Langel et al. 2009). Red mark syndrome, also named cold-water erythema disease, causes skin problems in rainbow trout (*Oncorhynchus mykiss* Walbaum) at low temperatures

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Proteomic analysis of epidermal mucus from sea lice-infected Atlantic salmon, *Salmo salar* L.

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¹International Research Institute of Stavanger (IRIS), Stavanger, Norway; ²Stavanger Aquaculture Research Centre, Stavanger, Norway; ³Norwegian QA - QA Service, Mandal, Norway; ⁴The Norwegian Multiple Sclerosis Centre, Department of Neurology, Haukeland Hospital, Bergen, Norway; ⁵KG Jhaan Multiple Sclerosis Centre, Department of Clinical Medicine, University of Bergen, Norway

Abstract
Health diets that contain immunostimulants and other functional ingredients can strengthen the immune response in Atlantic salmon, *Salmo salar*, and thereby reduce sea lice, *Lepeophtheirus salmonis*, infection levels. Such diets can be used to supplement other treatments and will potentially reduce the need for dosing and medication. A sea lice infection trial was conducted on fish with an average weight of 215 g. One control diet and four experimental diets containing functional ingredients were produced. The diets were fed to salmon for 4 weeks before infection with sea lice copepods. When fish had developed to challenge (L1/L2), 60 fish per diet were examined for sea lice. Mucus samples from fish fed the different diets were taken before and after lice infection. Mass spectrometry-based proteomics was used to characterize the protein composition in the epidermal mucus of Atlantic salmon and to identify quantitative differences in protein expression. Multivariate analysis of the generated data was performed to identify protein biomarkers. Protein biomarkers associated with functional feed intake and with sea lice infection have been identified and can form the basis for strategic selection experiments with selected functional feeds.

Keywords: Atlantic salmon, epidermal mucus, mass spectrometry, proteomics, sea lice, biomarker

Introduction
Sea lice, *Lepeophtheirus salmonis*, infection is one of the biggest challenges faced by the aquaculture industry in the North Atlantic region. The sea lice cause a substantial loss to the industry every year, and the results of sea lice infection from farmed fish to wild fish is of great concern. Health diets are becoming a more important part of the integrated pest management strategy against sea lice. The risk of developing resistance against sea lice medication and the difficulties in applying such treatments to large cages have greatly increased the focus on functional feed ingredients in aquaculture. Some immunostimulants or functional ingredients have been shown to have an effect against sea lice (Shaw, Williams & Torst 2010; Kefauver et al. 2010; Range et al. 2010), but the mechanism behind this effect is not fully understood. Fish mucus is a complex material and functions as a mechanical protection against the environment. It also contains enzymes, antibodies and other immune-related compounds (Fink, Johnson & Rose 2006; Torst et al. 2010; Torst & Rose 2010). Mucus is seen as a promising biological matrix for biomarker identification given its ease of sampling.

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Effect of temperature and diet on wound healing in Atlantic salmon (*Salmo salar* L.)

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Abstract
Compromised skin integrity of farmed Atlantic salmon, commonly occurring under low temperature and stressful conditions, has major impacts on animal welfare and economic productivity. Even fish with minimal scale loss and minor wounds can suffer from secondary infections, causing downgrading and mortalities. Wound healing is a complex process, where water temperature and nutrition play key roles. In this study, Atlantic salmon (200 g) were held at different water temperatures (4 or 12 °C) and fed three different diets for 10 weeks before artificial wounds were inflicted and the wound healing process monitored for 2 weeks. The fish were fed either a control diet, a diet supplemented with zinc (Zn) or a diet containing a combination of functional ingredients in addition to Zn. The effect of diet was assessed through subjective and quantitative skin histology and the transcription of skin-associated chemokines. Histology confirmed that wound healing was faster at 12 °C. The epidermis was more organized, and image analysis of digitized skin slides showed that fish fed diets with added Zn had a significantly larger area of the epidermis covered by mucus cells in the deeper layers after 2 weeks, representing more advanced healing progression. Constitutive levels of the newly described chemokines, herein named CK 11A, B and C, confirmed their preferential expression in skin compared to other tissues. Contrasting modulation at 4 and 12 °C were seen for all three chemokines during the wound healing time course, while the Zn-supplemented diets significantly increased the expression of CK 11A and B during the last 24 h of the healing phase.

Keywords: Chemokine, Wound healing, Atlantic salmon, Mucus cells, Quantitative histology

Introduction
Impaired skin integrity is common in Atlantic salmon (*Salmo salar* L.), farming, especially during transfer to seawater and at low water temperatures. In addition to alterations in environmental conditions, intensively farmed fish are inevitably exposed to a variety of stressors including temperature, grading, vaccination and infections, again that can potentially

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UNIVERSITY OF ABERDEEN

UNIVERSITY OF STIRLING

IRIS

International Research Institute of Stavanger

INIA

Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

HELSE STAVANGER

Stavanger universitetssjukehus

SKRETTING

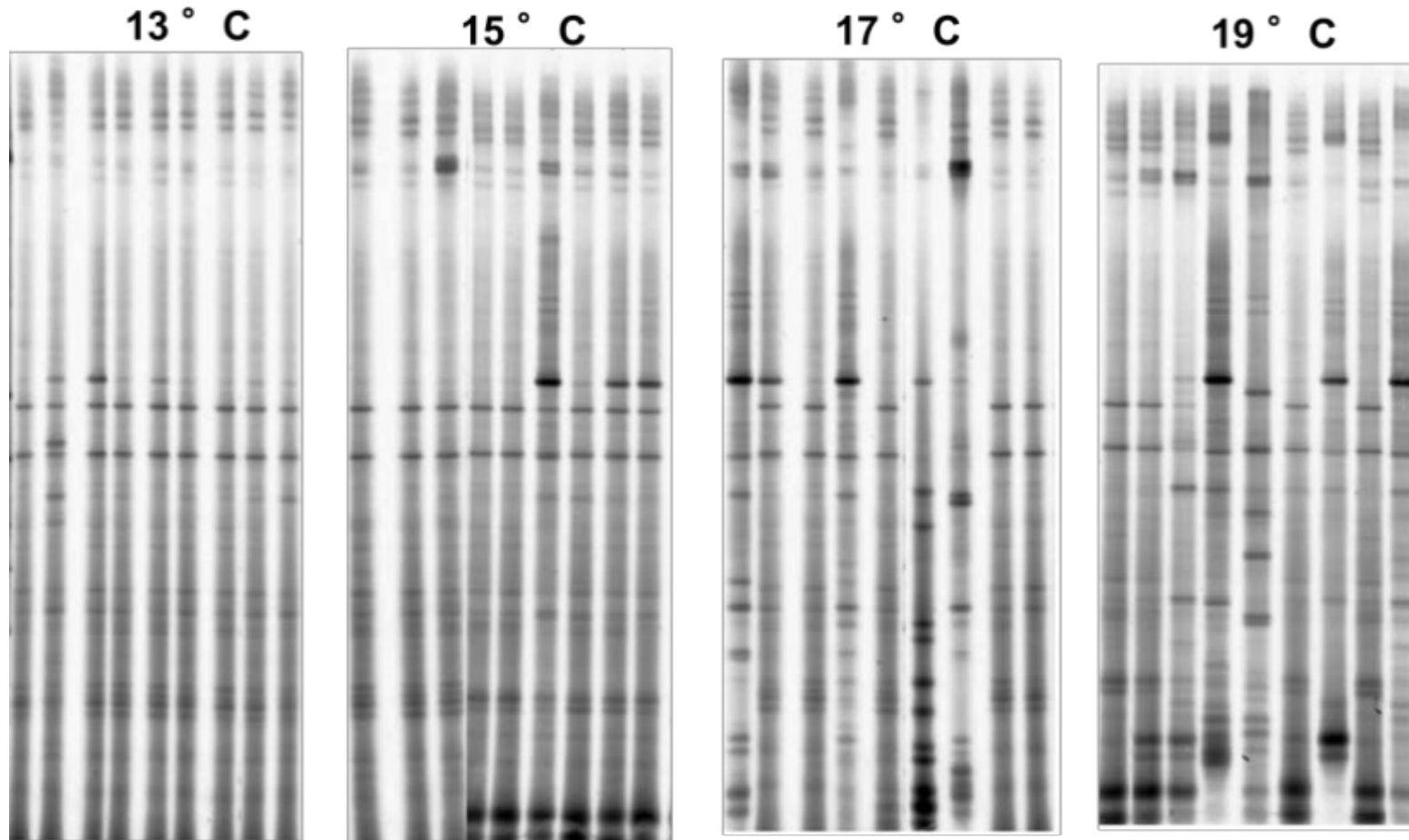
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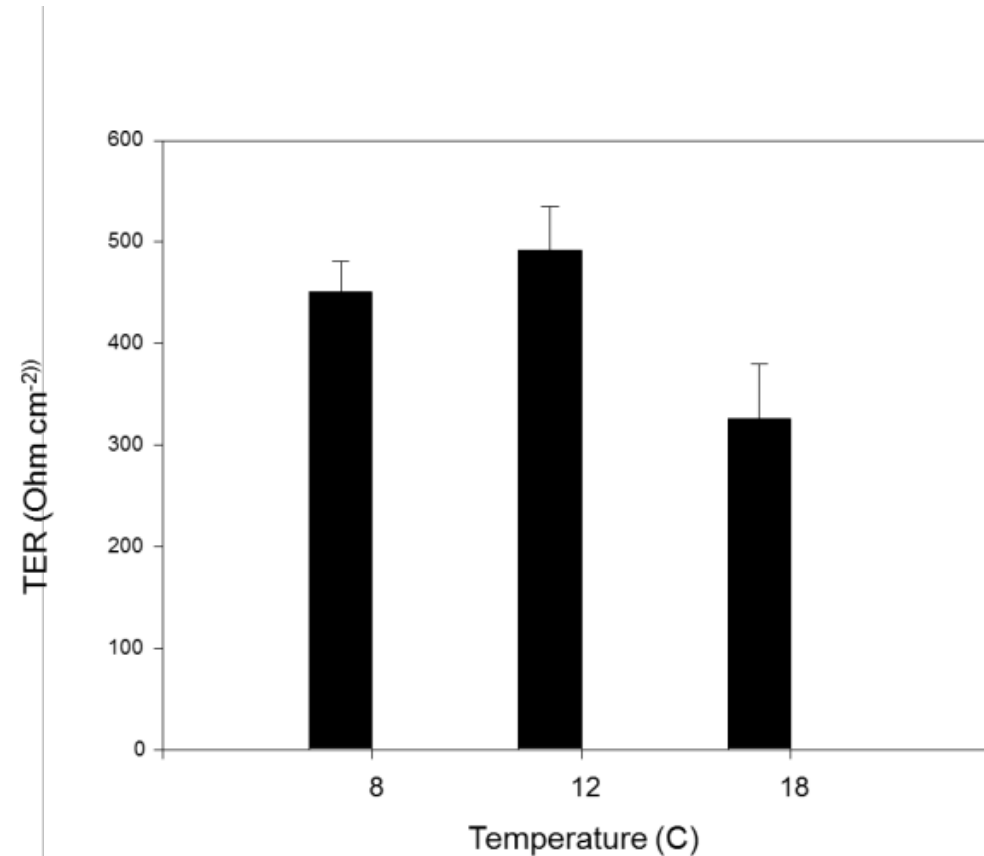
Novel technologies to investigate the role of functional diets

Microbiota disruption in Atlantic salmon



McGurk at al., unpublished data

Increased gut permeability in Atlantic salmon above 18°C

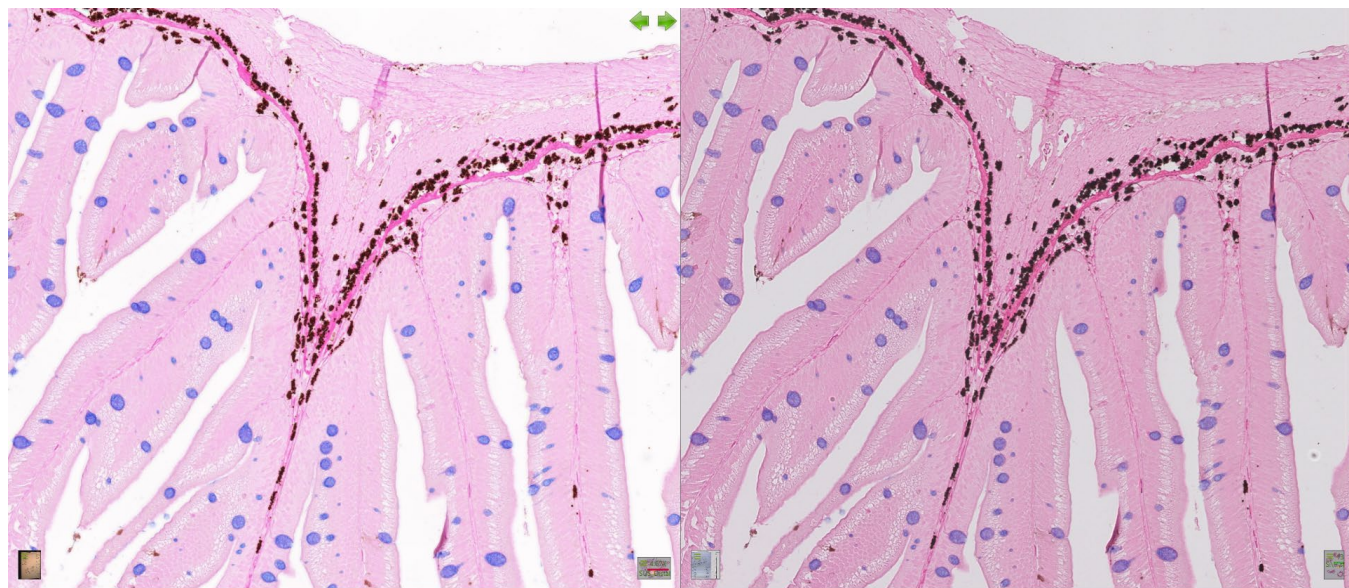


TER-Transepithelial resistance

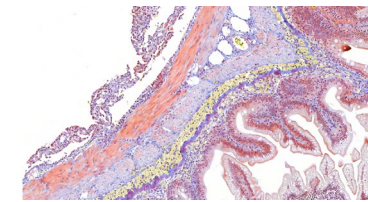
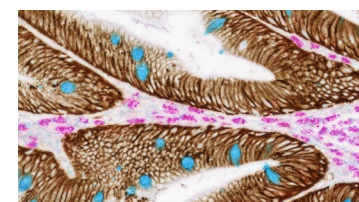
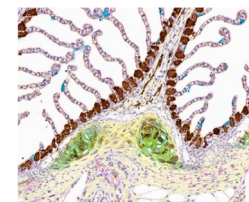
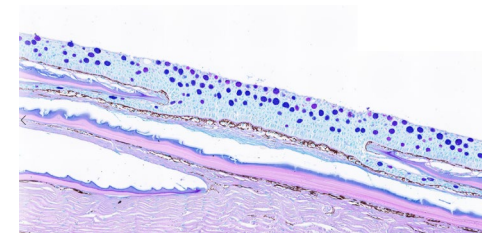
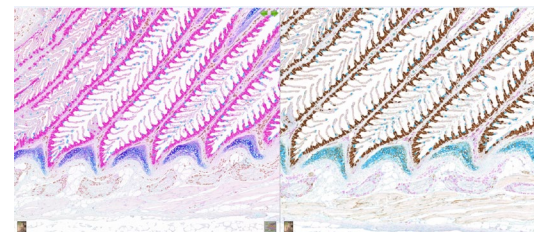
Fontanillas et al., 2008, XIII- International Symposium on Fish Nutrition and Feeding

Histology

Study of the microanatomy of cells, tissues, and organs as seen through a microscope

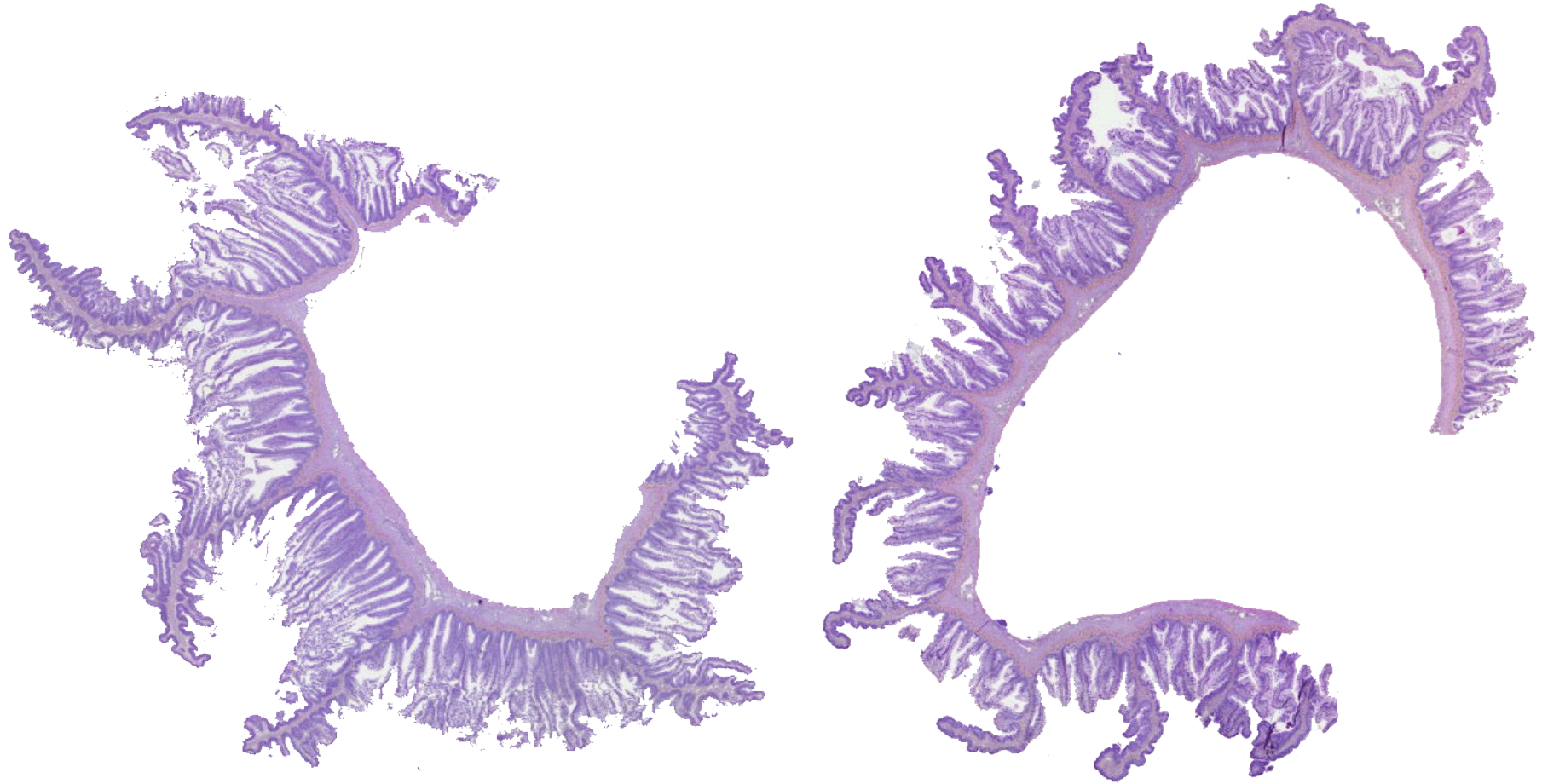


**New possibilities
with new equipment**



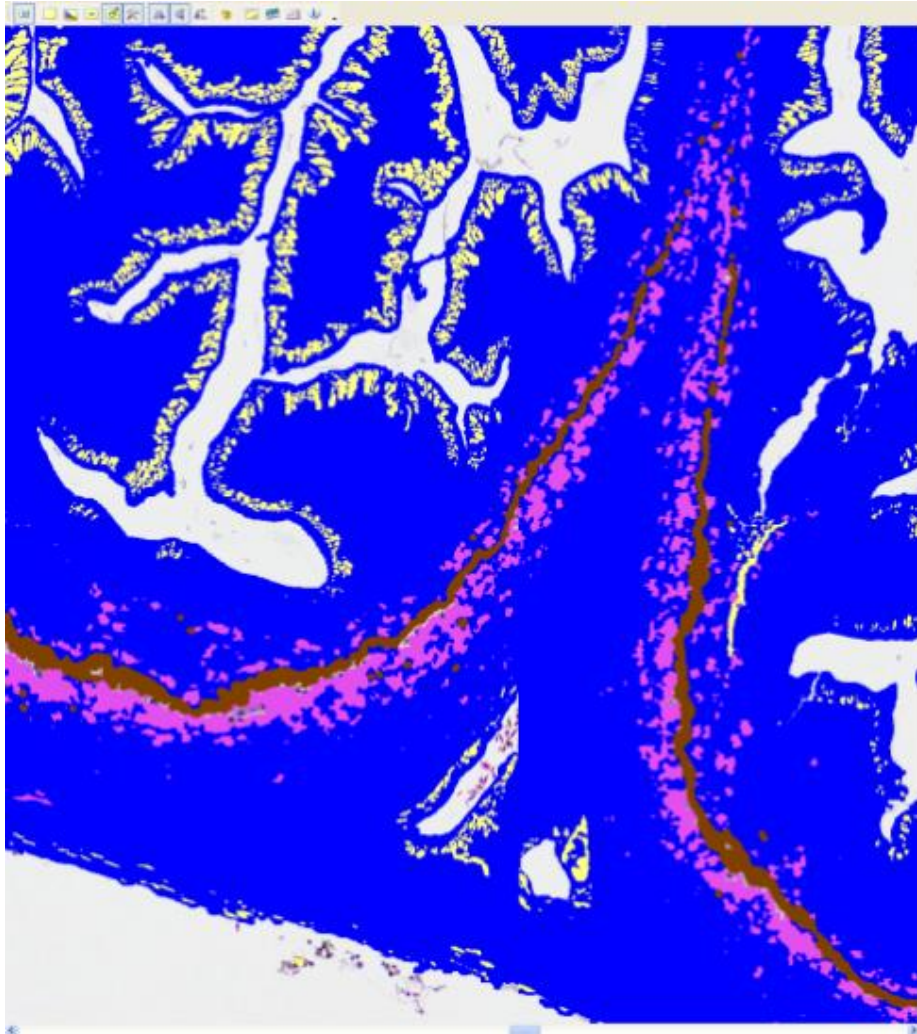
Gut stabilization

Histological indicators of gut inflammation

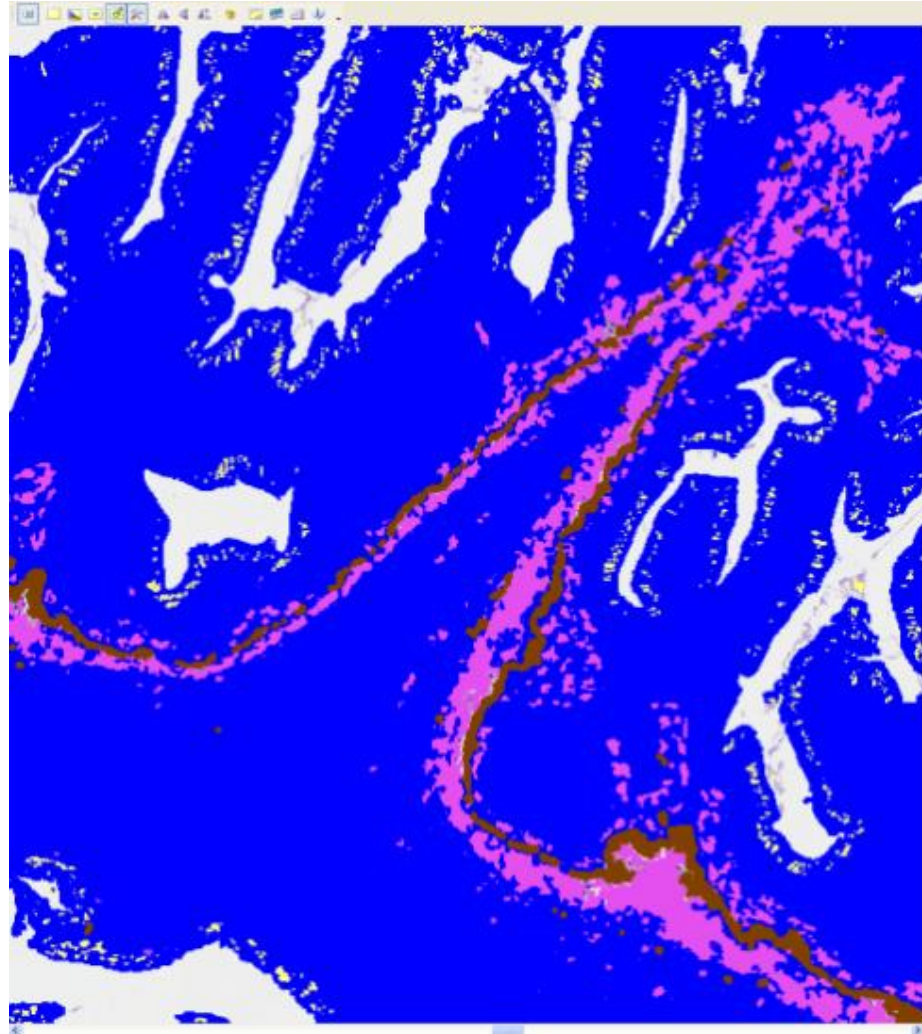


Gut stabilization

Histological indicators of gut inflammation



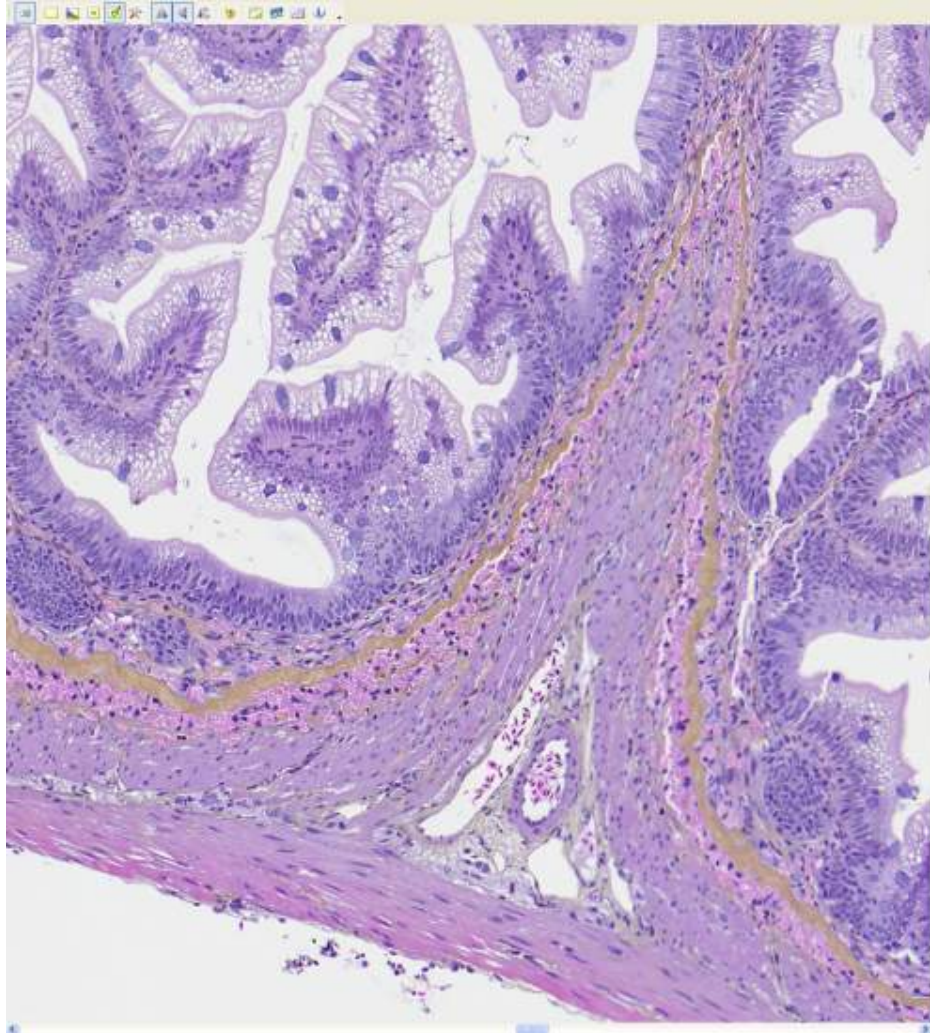
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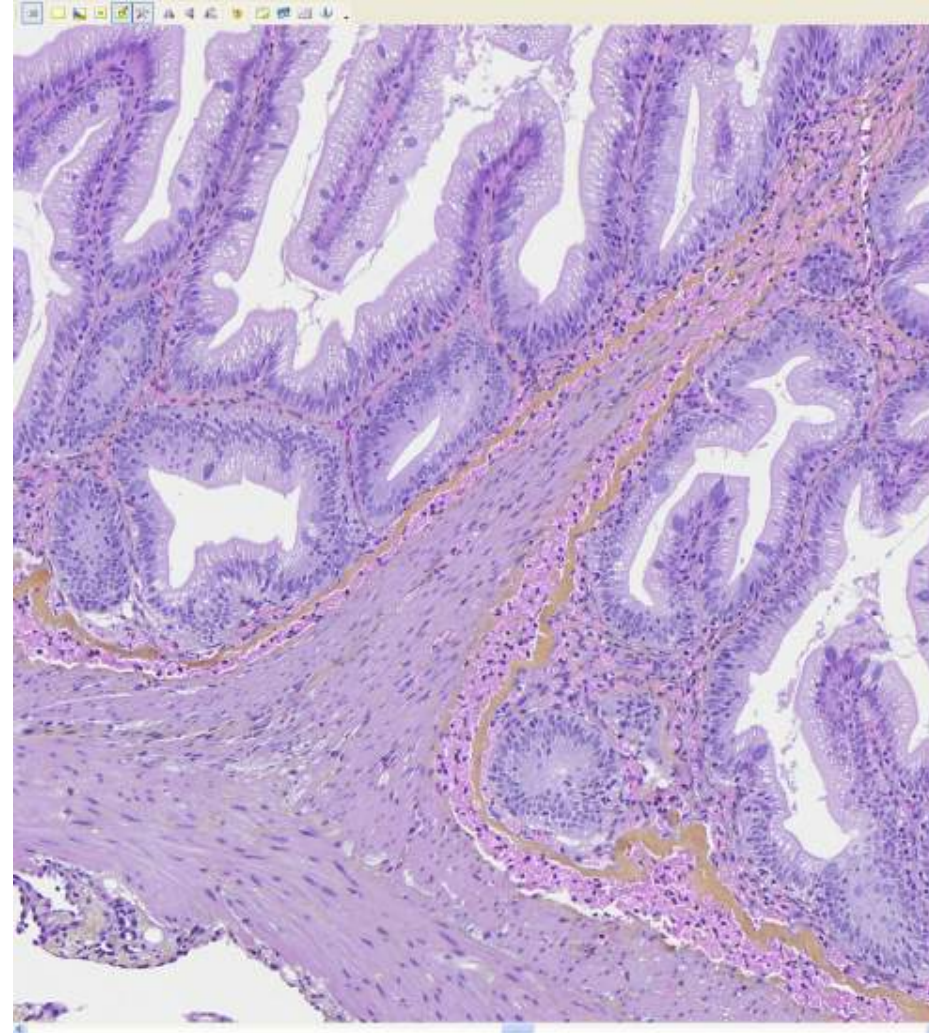
Control diet: 18°C

Gut stabilization

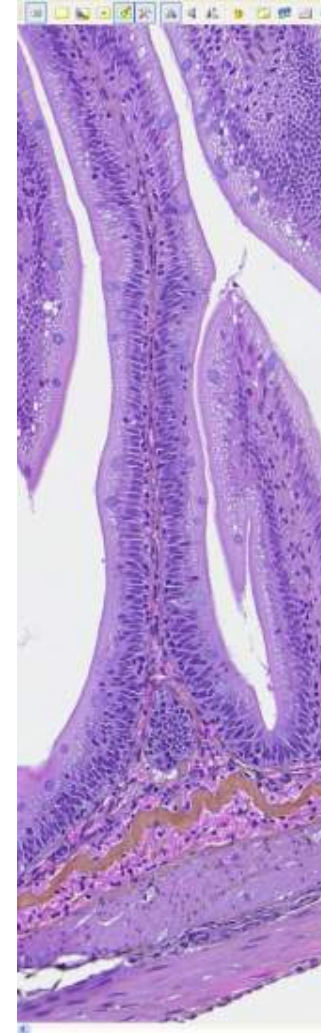
Histological indicators of gut inflammation



Control diet: 12°C

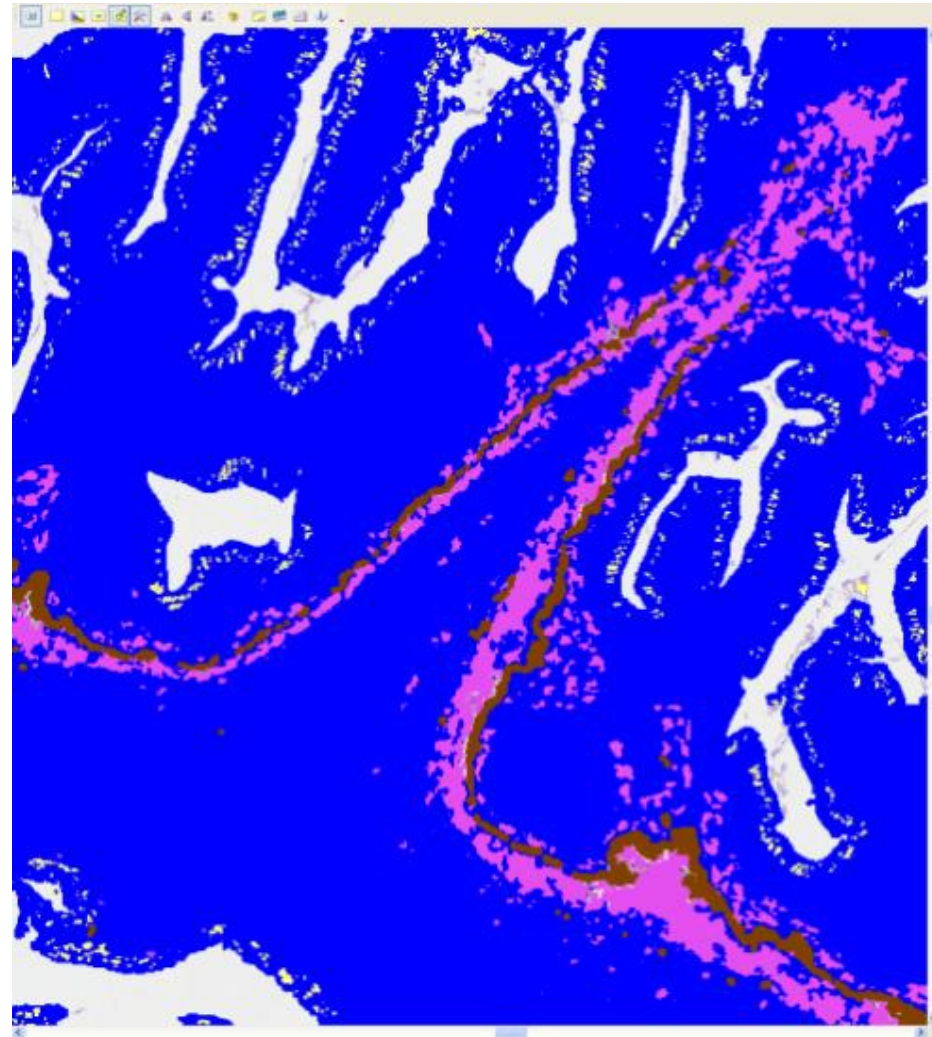


Control diet: 18°C

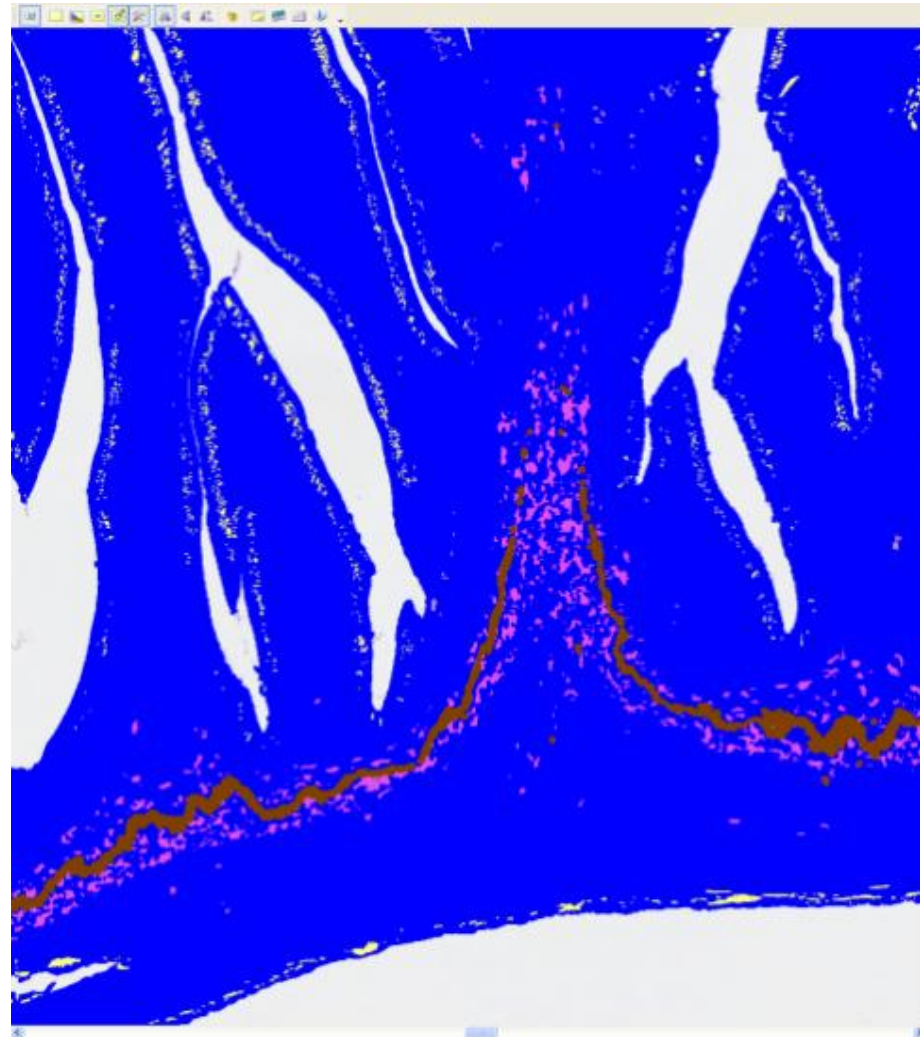


Gut stabilization

Histological indicators of gut inflammation



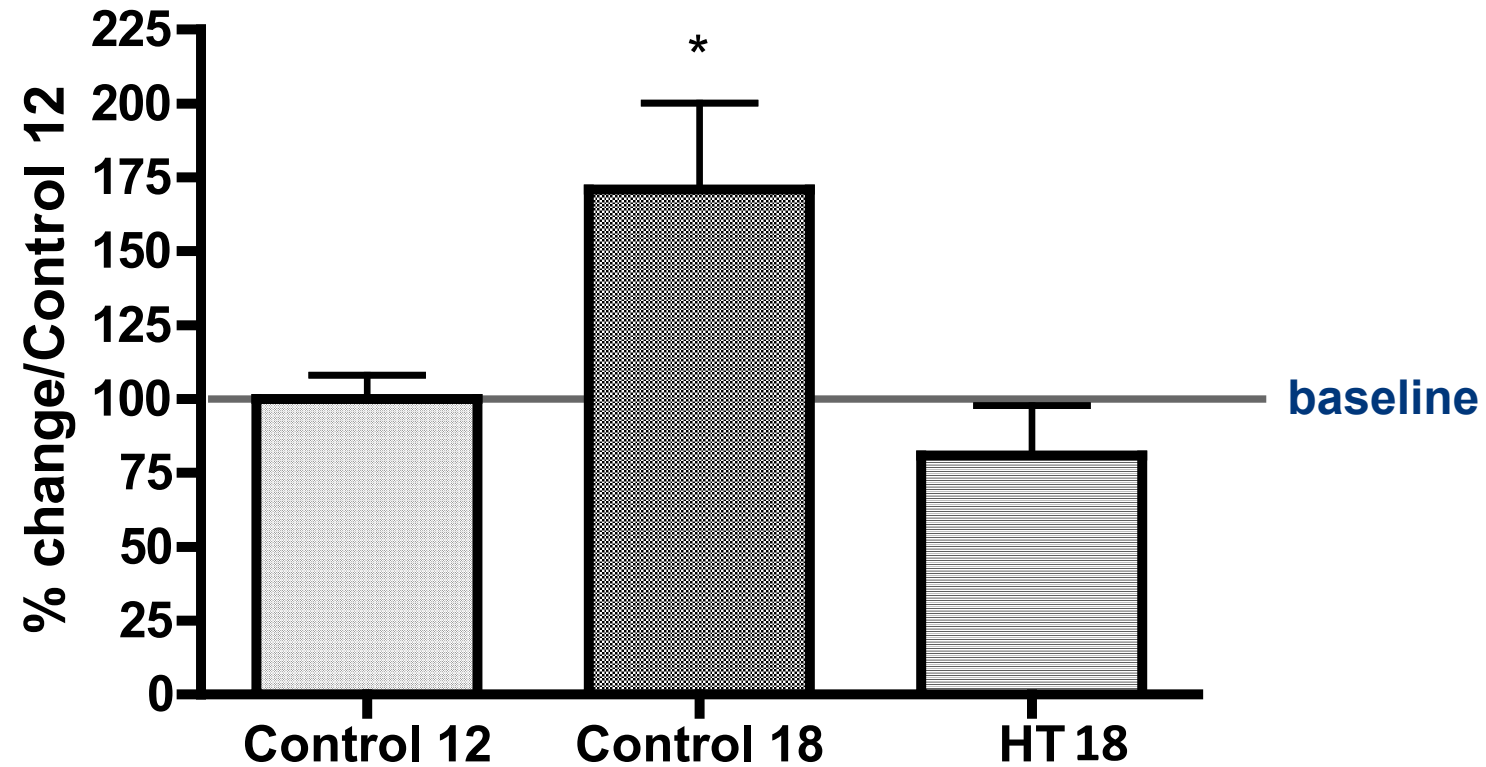
Control diet: 18°C



HT: 18°C

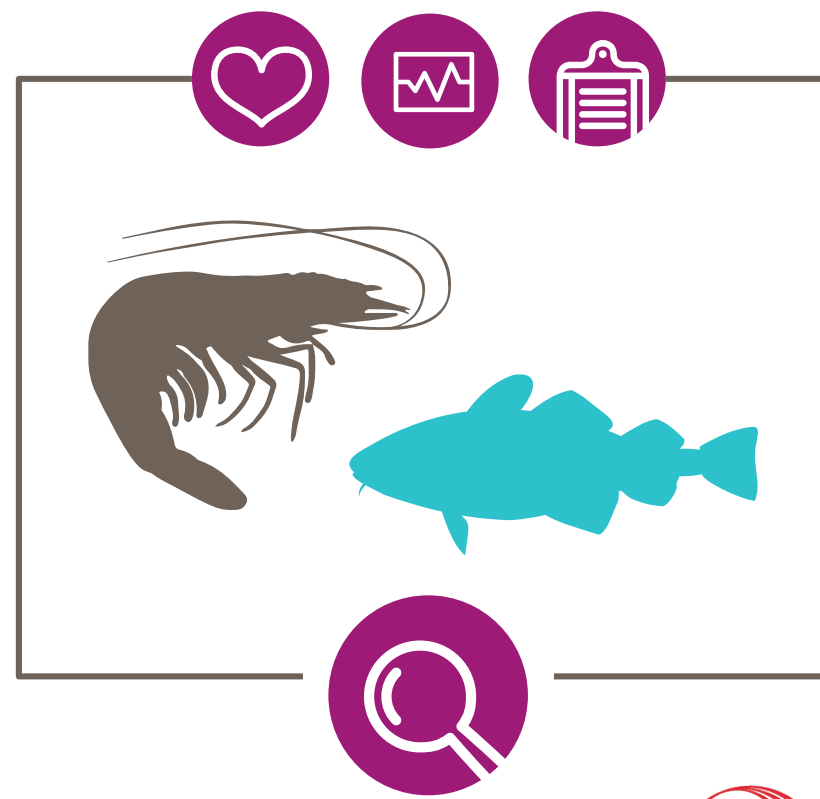
Gut stabilization

Histological measurement of inflammatory gut cells



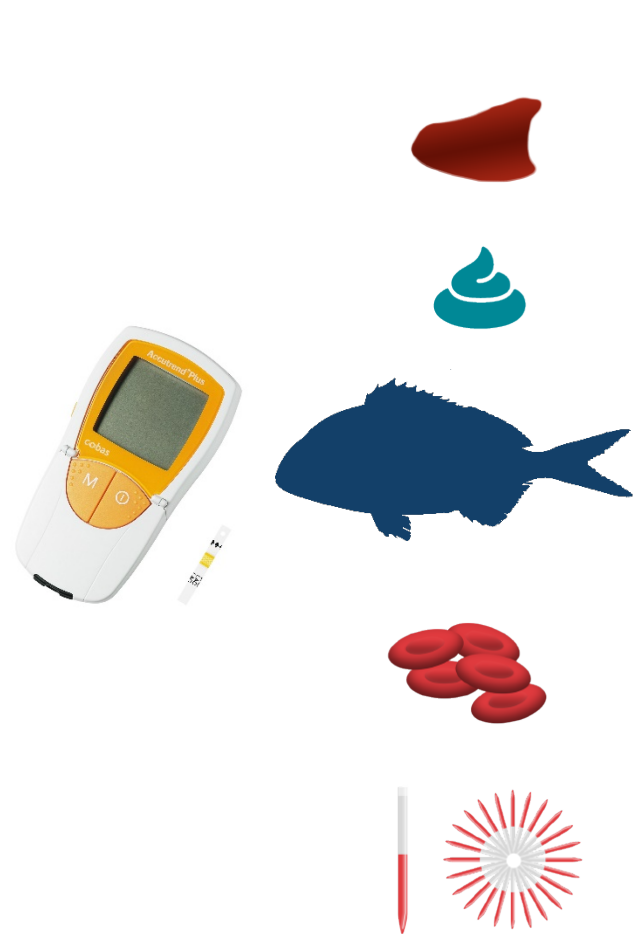
Rapid analytical tools

Development of diagnostic tools to assess rapidly fish/shrimp health

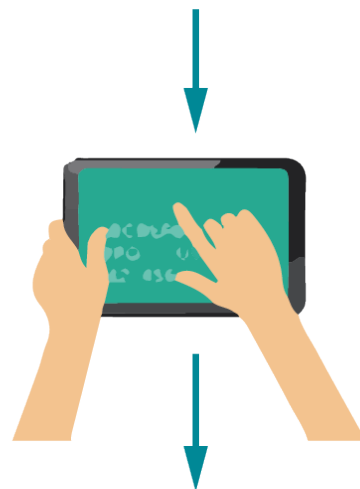


Rapid analytical tools - opportunities

Biological



Analyses



Decision making

Chemical



Summary

- Disease and environmental stress comprise fish health and welfare
- Functional nutrition can play a role in mitigating health challenges
- Supporting natural defence mechanisms can reduce reliance on drug treatments and resultantly antimicrobial resistance development
- Novel technologies are employed to reveal the modes of action and functionality of potential novel dietary characteristics
- Rapid diagnostic tools have the potential for optimising prevention and control strategies