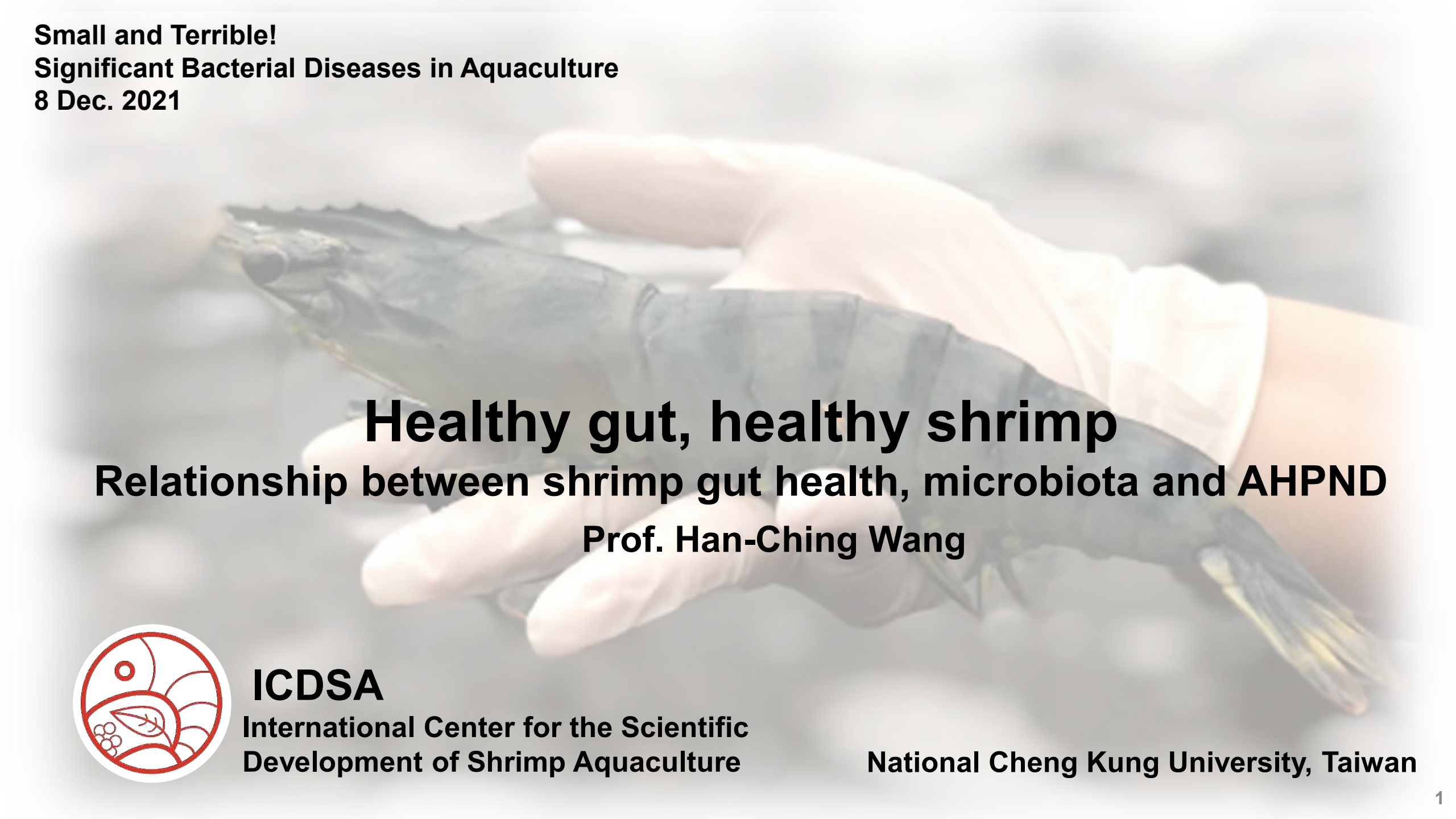
Small and Terrible!
Significant Bacterial Diseases in Aquaculture
8 Dec. 2021



Healthy gut, healthy shrimp

Relationship between shrimp gut health, microbiota and AHPND

Prof. Han-Ching Wang

ICDSA
International Center for the Scientific Development of Shrimp Aquaculture

National Cheng Kung University, Taiwan

- Shrimp farming - a major global industry with 70% of production in Asian countries
- Matching with the United Nations Sustainable Development Goals:

- Biggest threats –
  - Disease problems
  - Lacking source of good stock and fry
  - Aquaculture environment
- The urgency of solving the epidemic problem in Taiwan's agricultural and fishery industries

- **Reduce shrimp farming loss caused by extreme weather**
- **Increase shrimp production potential**
- **Help meet food demand in a growing population**

Trends in Ecology & Evolution

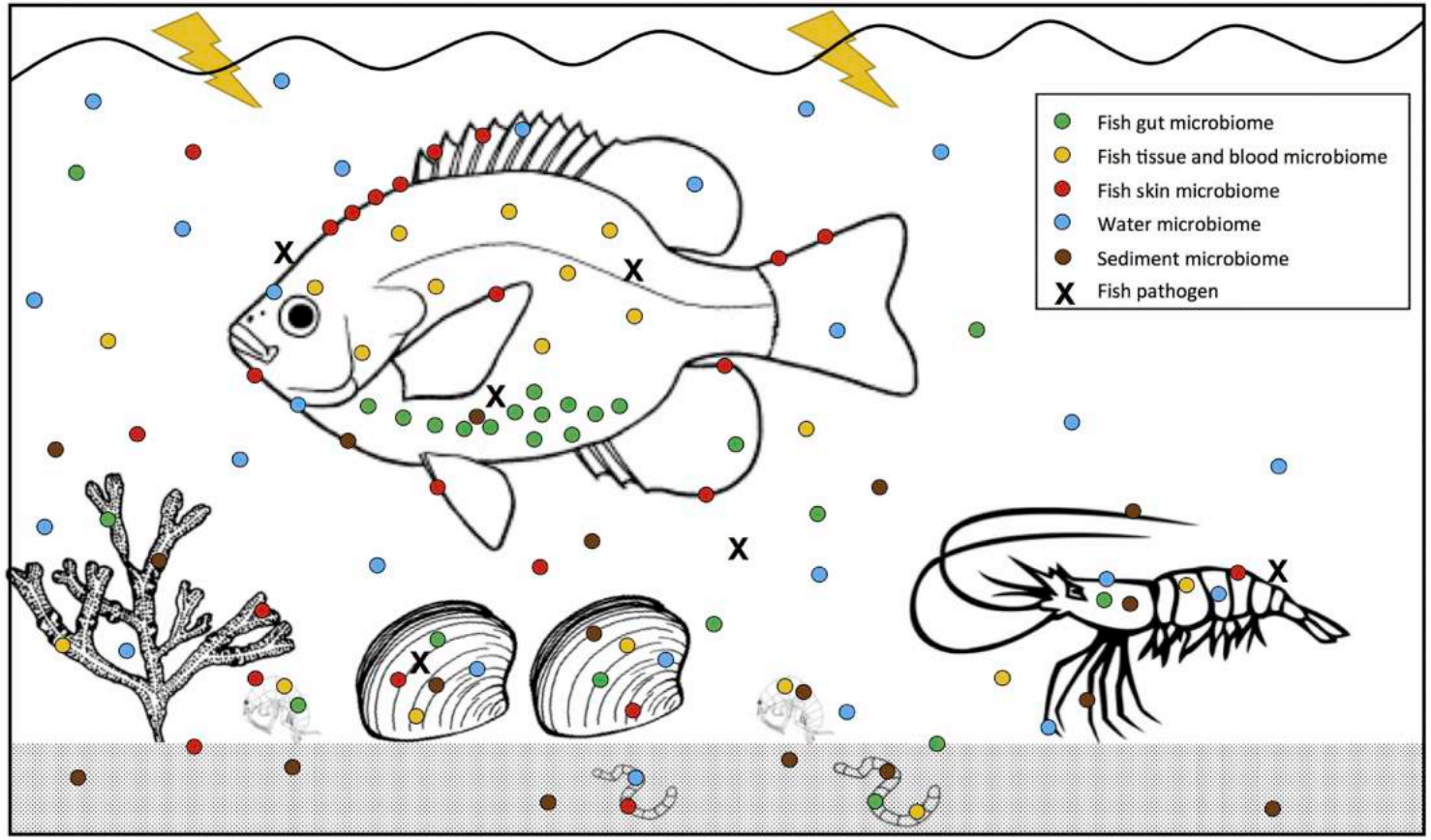
CellPress REVIEWS

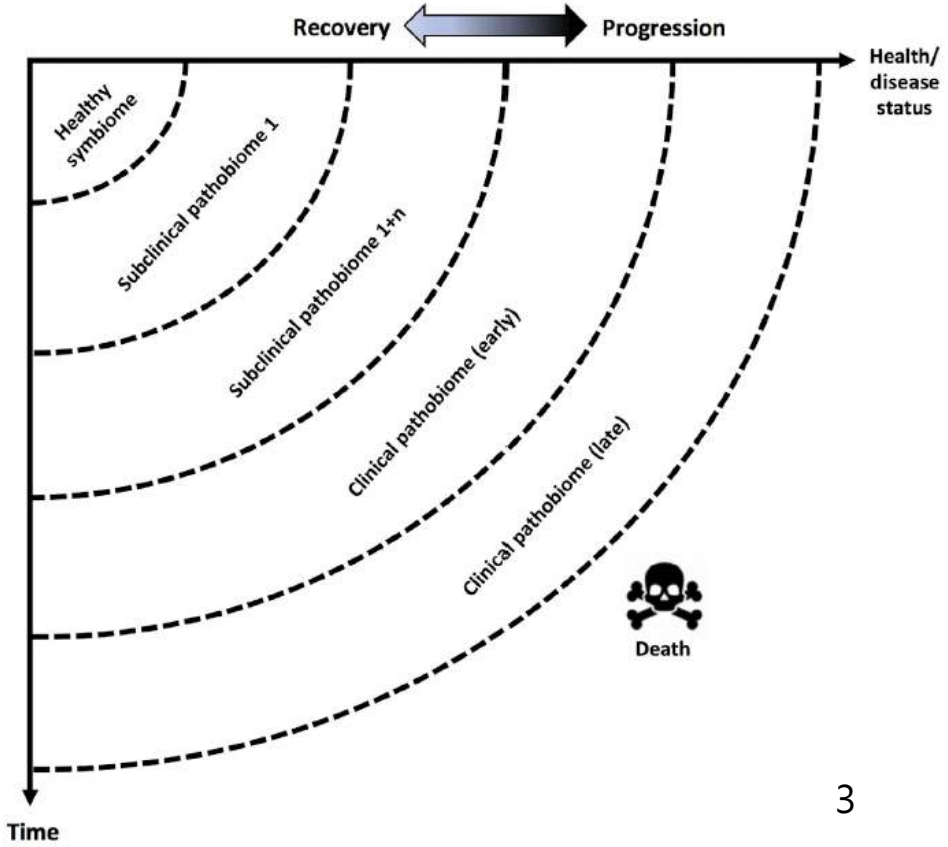
Review

The Pathobiome in Animal and Plant Diseases

David Bass,[1,2,3,@,*] Grant D. Stentiford,[1,2,@] Han-Ching Wang,[4,5,@] Britt Koskella,[6,@] and Charles R. Tyler[2,7]

Microbial complexity in a typical host–symbiont–environment system





Gut Health

- **Gut (also called Gastrointestinal or GIT) functionality and health**
  - Effective function
    - important factor in determining animal performance
  - Complex mechanisms

- **Not only for nutrient absorption**
  - also related to many physical responses

- **Improved understanding in digestive physiology and dietary requirements**
  - lead to significant gains in productive performance of farmed animals

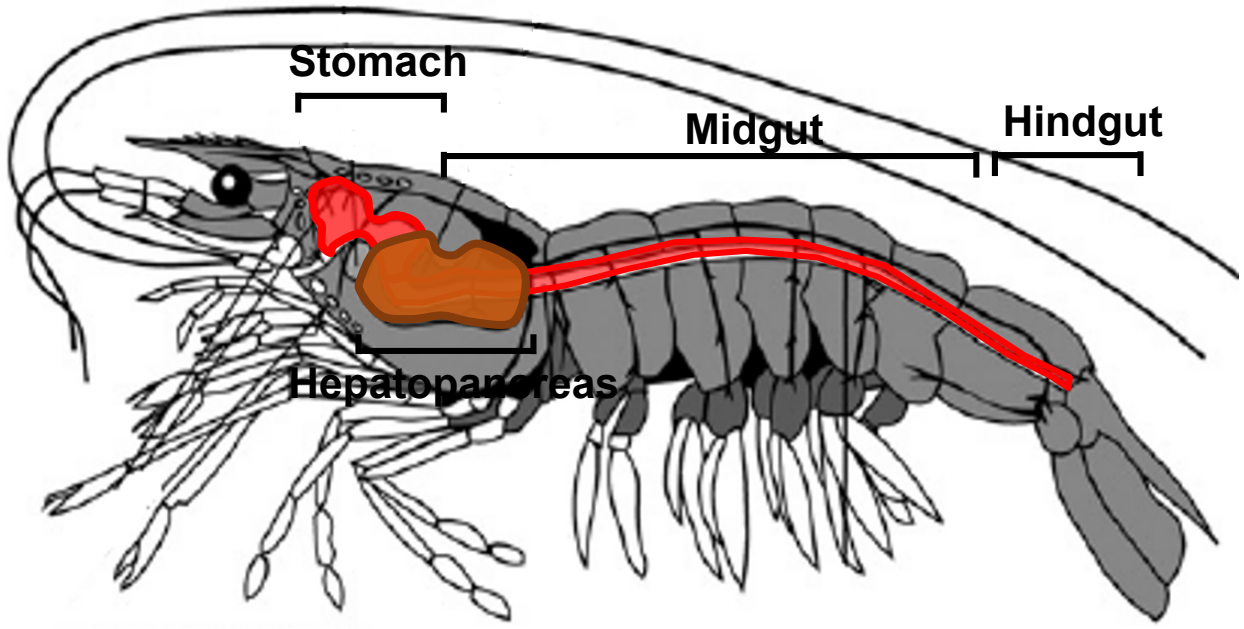
Gastrointestinal (gut) health

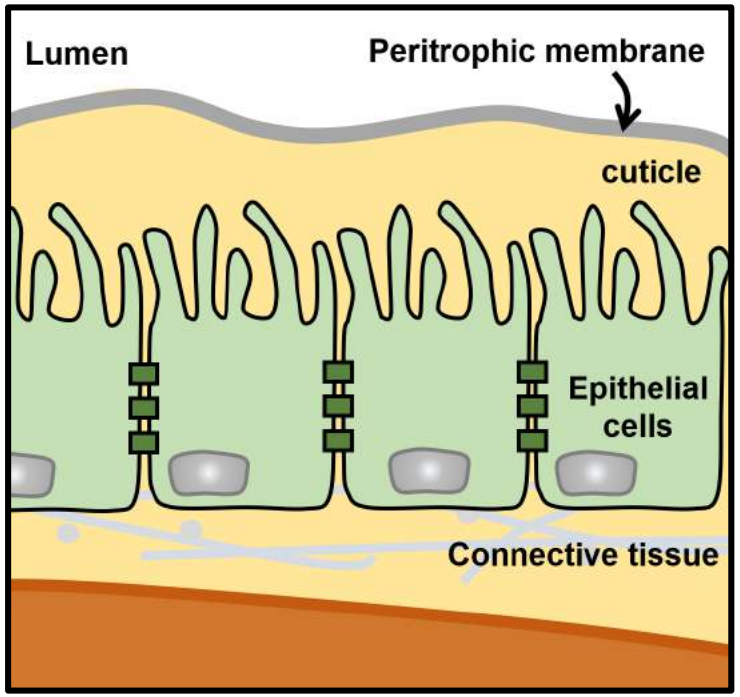
an increasingly important topic in animal nutrition and health

Celi *et al.*, 2017

Shrimp digestive tract

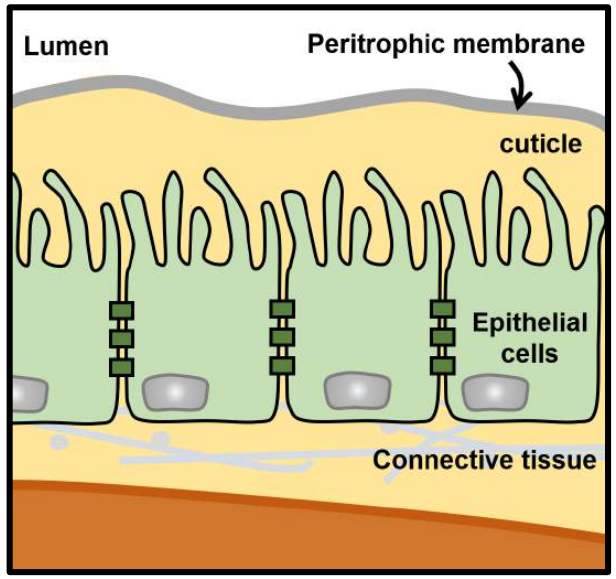
- **Shrimp digestive tract**
  - **Foregut [stomach]**
  - **Hepatopancreas**
  - **Midgut [anterior and posterior caecum]**
  - **Hindgut [posterior caecum to anus]**



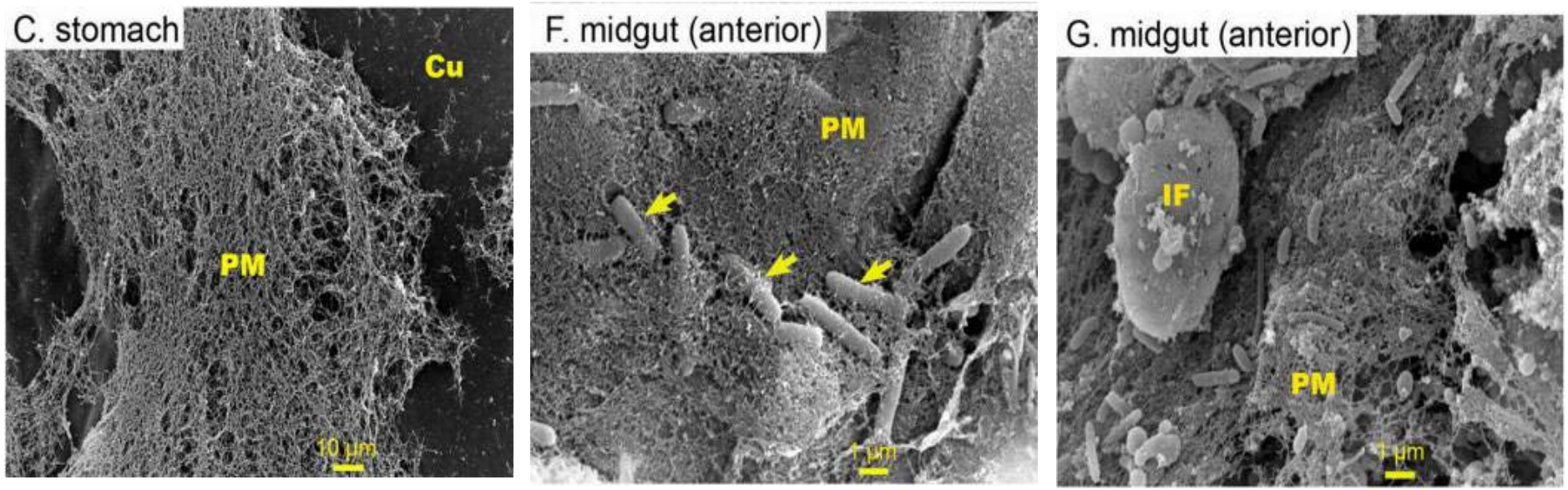


- **Structure of shrimp gut**
  - **Peritrophic membrane**
    - lies between the lumen and epithelia
    - protects the ECs from abrasive particles and pathogens.
  - **Cuticle layer**

Shrimp digestive tract



Presence of normal flora in the GI tract of pond-cultured *Penaeus monodon*



Peritrophic membrane (PM), cuticle (Cu), ingested food (IF) (Source: Soonthornchai *et al.*, 2015)

Microbiome dynamics and health

Microbiota
Microbiome

- **The health of an animal**
  - highly associated with microbiota
    - the composition and function of a microbial community inside of the intestinal system and outside of the body
    - contribute nutrients and energy to the host
    - in the training and development of immune system
- **A microbiota balance is maintained with**
  - the host factors and environmental factors

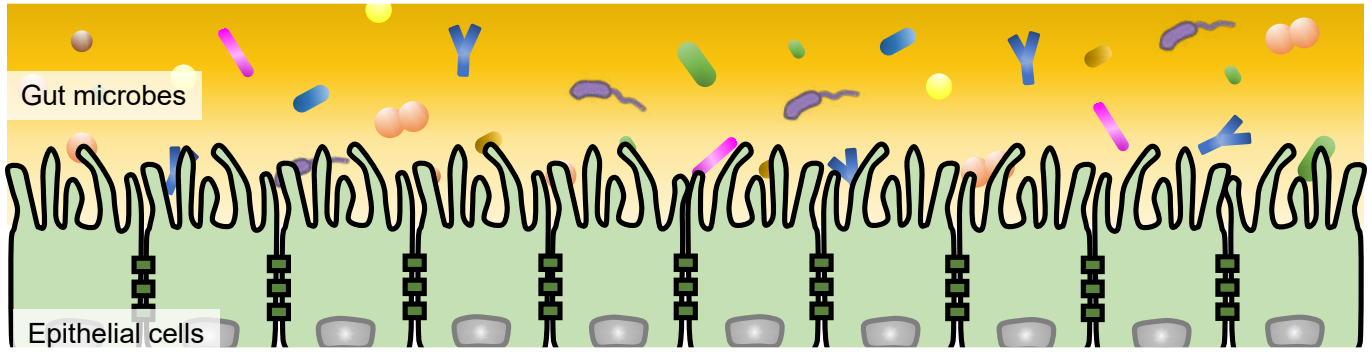
Fassarella M, Blaak EE, Penders J, et al.
Gut 2021;70:595–605.

Factors determining gut microbiome stability

- **Resilience of the gut microbiome seems to be governed by**

(A) characteristics of the gut microbiome

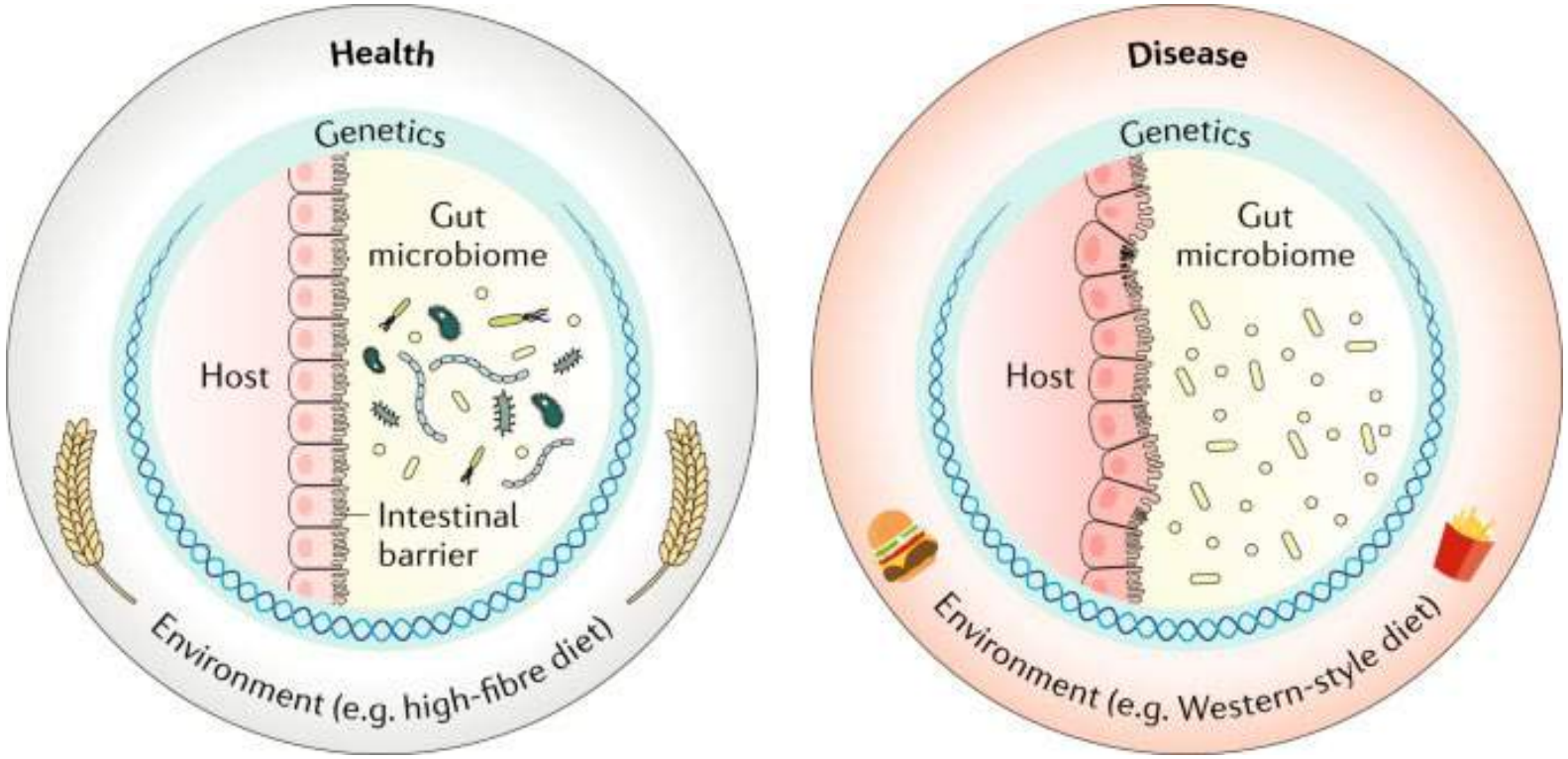
(B) host control



Fassarella M, Blaak EE, Penders J, et al.
Gut 2021;70:595–605.

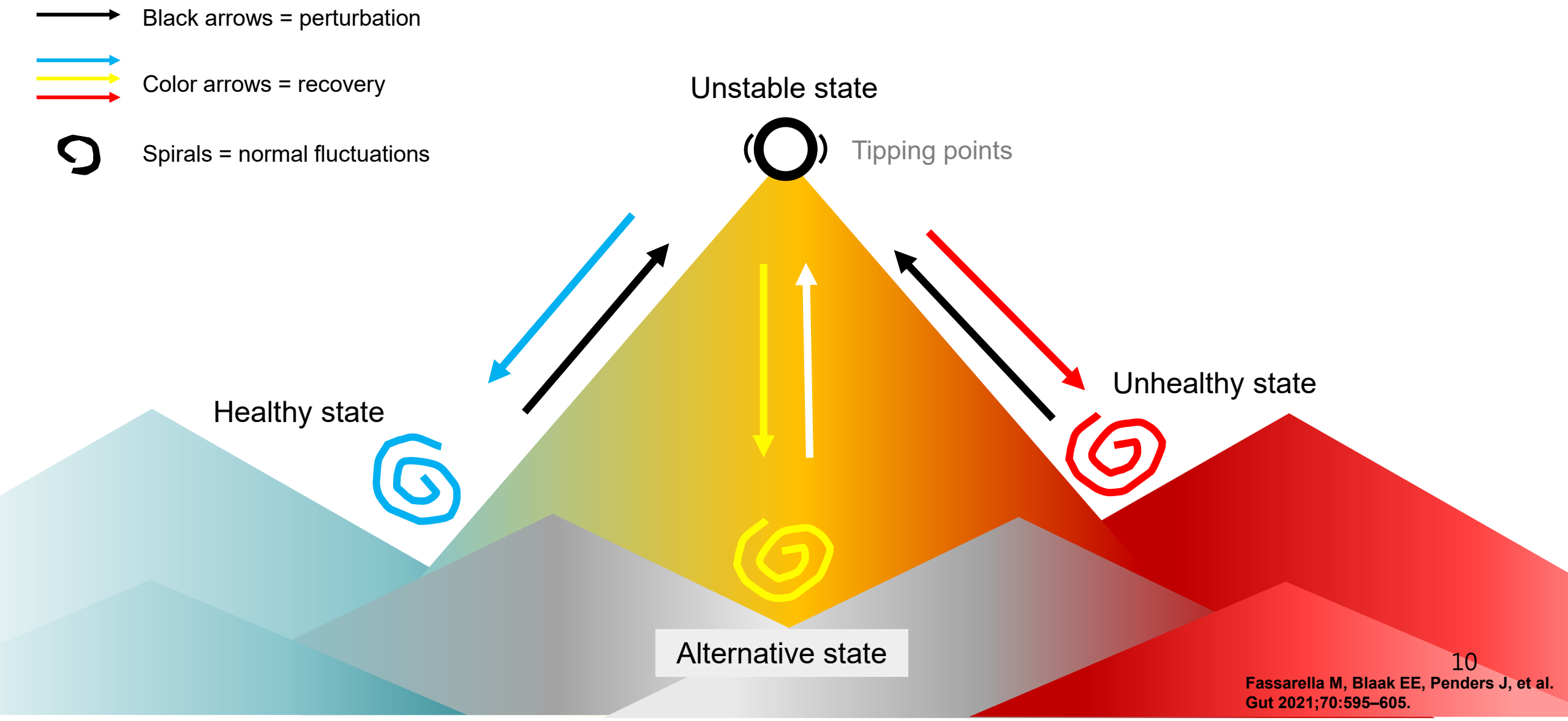
Establishing the role of diet in the microbiota–disease axis

- Environmental factors and their influence on the gut microbiota



Nature Reviews Gastroenterology & Hepatology volume 16, pages86–87 (2019)

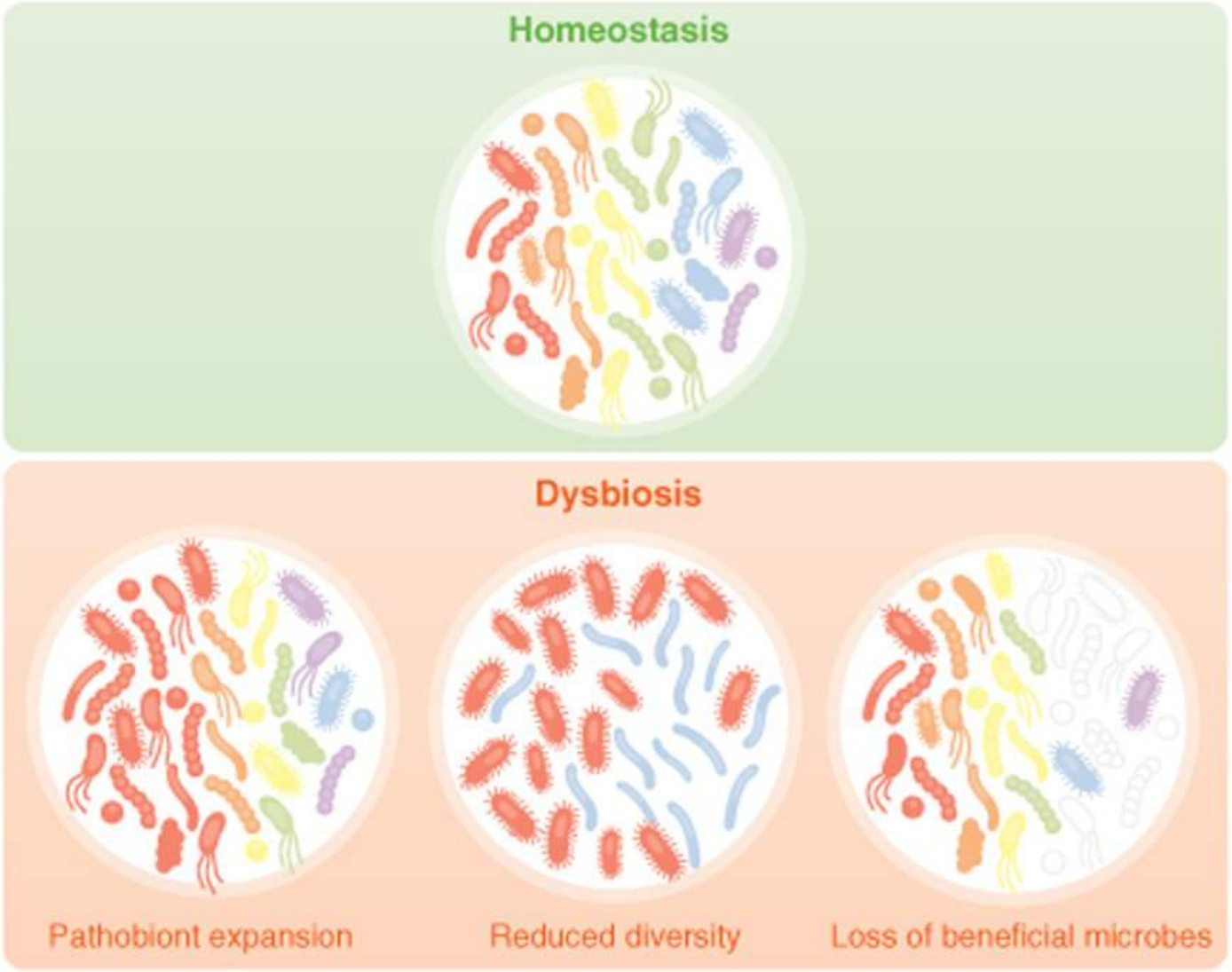
Gut microbiome resilience landscape



Fassarella M, Blaak EE, Penders J, et al.
Gut 2021;70:595–605.

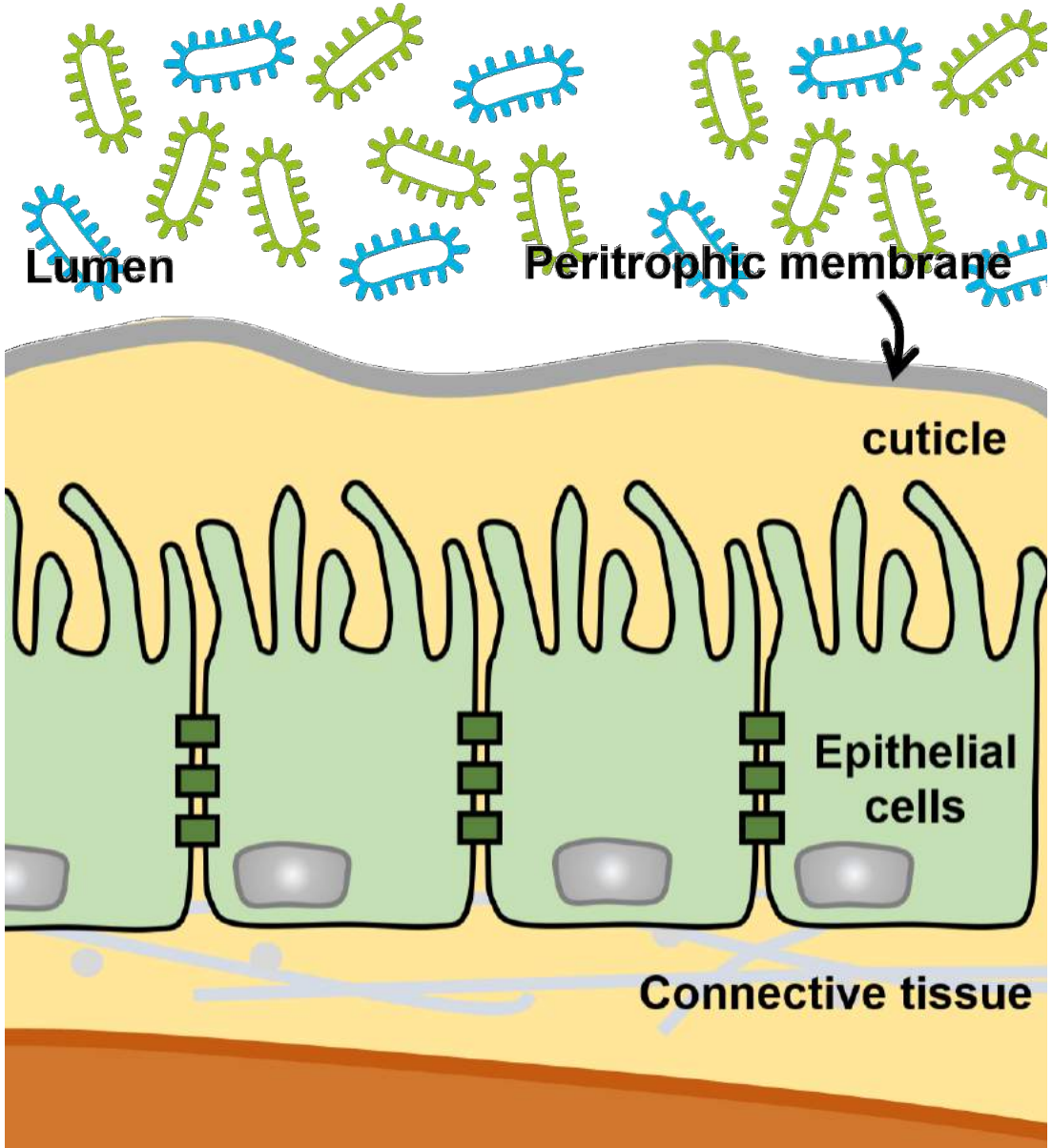
Dysbiosis

- **Dysbiosis**
  - A loss of beneficial microbes
  - Expansion of pathobionts
  - Loss of diversity
- **Effects of dysbiosis**
  - alter nutrient availability to stomach bacteria
  - impact their metabolic function



Cellular Microbiology, Volume: 16, Issue: 7, Pages: 1024-1033, First published: 05 May 2014, DOI: (10.1111/cmi.12308)

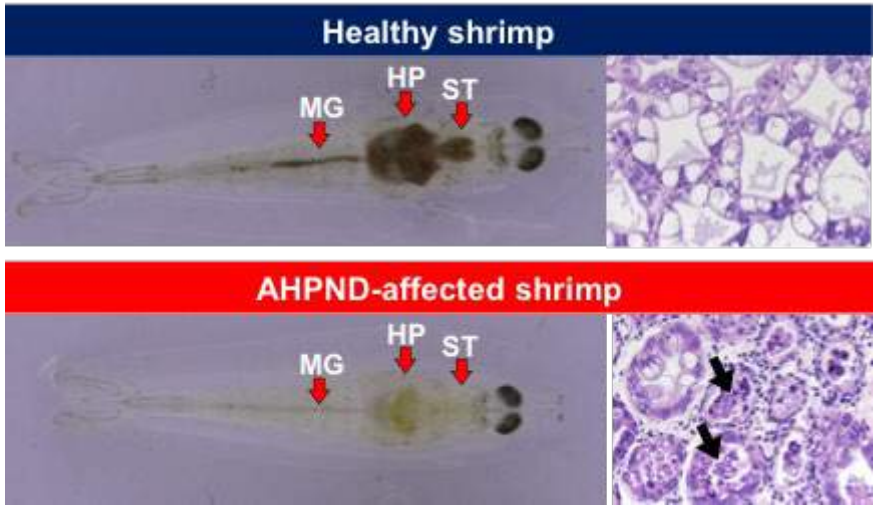
Shrimp digestive tract and diseases

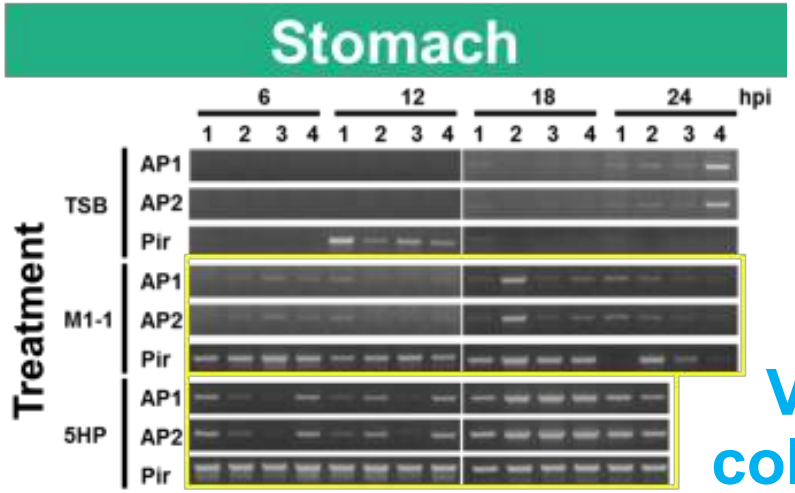


- **Key factors**
  - Microbiota
  - Stomach metabolism
  - Biofilm formation
  - Peritrophic membrane
  - Cuticle
  - Immune factors
  - Epithelial cells
  - Hepatopancreas

Acute Hepatopancreatic Necrosis Disease

- **Acute Hepatopancreatic Necrosis Disease (AHPND)**
  - Clinical signs
    - Pale HP and empty stomach
    - Sloughing of HP tubule epithelial cells
  - **Pathogen – a virulent forms of *Vibrio parahaemolyticus* (virulent VP)**
    - Carries a specific plasmid
    - Contains **the PirA^{vp} and PirB^{vp} toxin** genes (Lee *et al*., 2015)
      - the major factor to damage the shrimp hepatopancreas

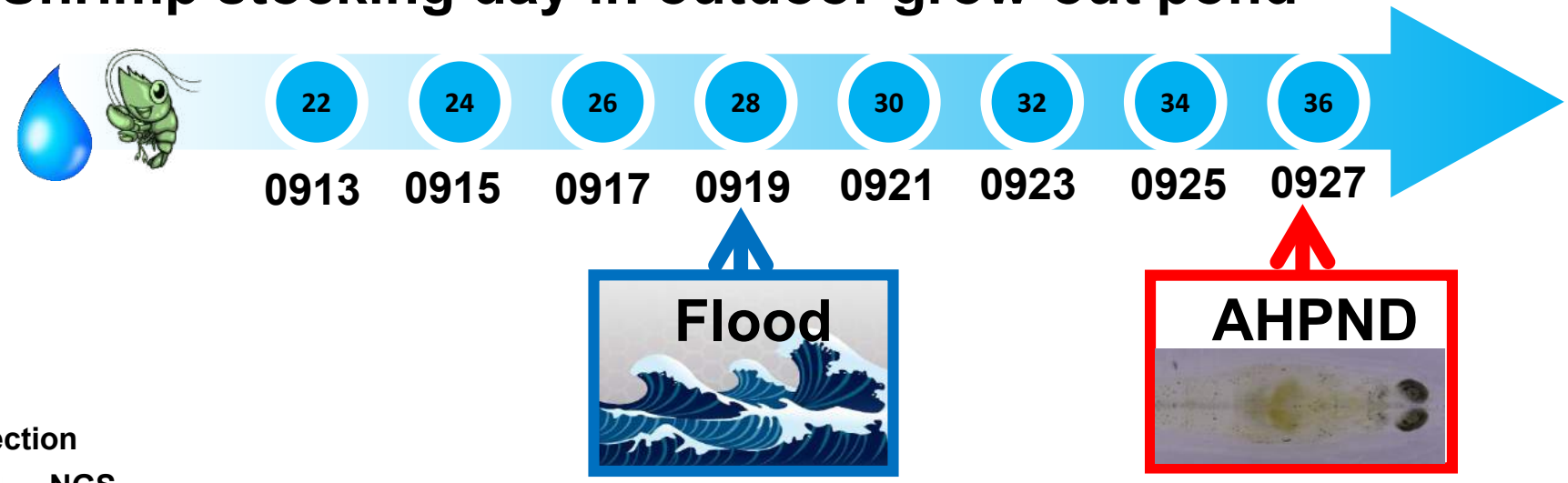


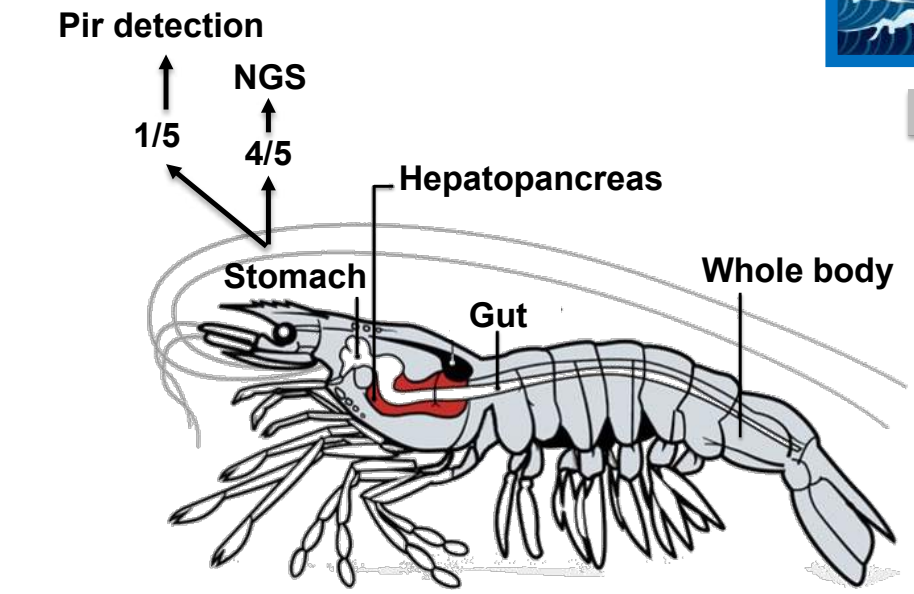


Virulent VP initially colonizes the stomach

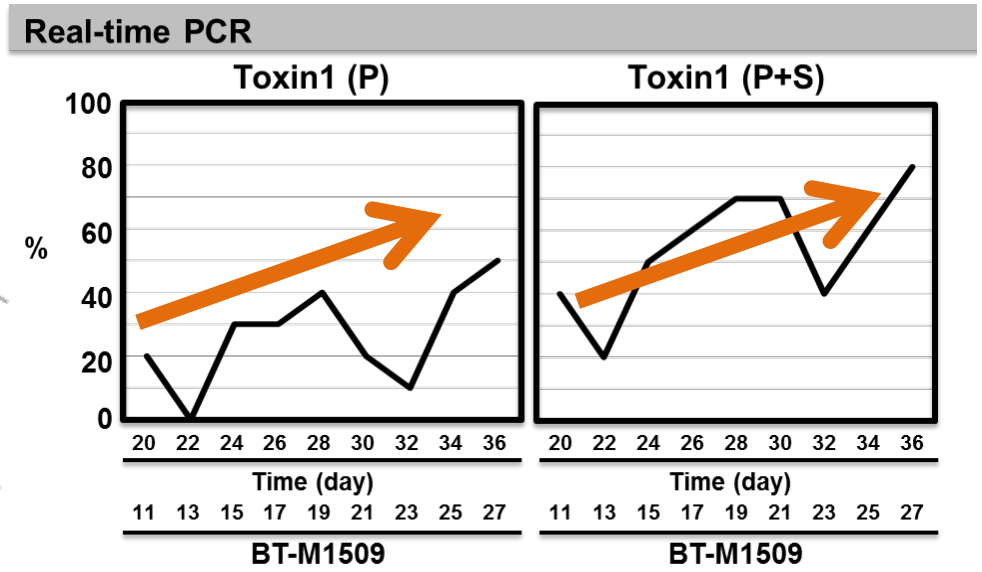
Sampling strategy for microbiome dynamic research

★Shrimp stocking day in outdoor grow-out pond

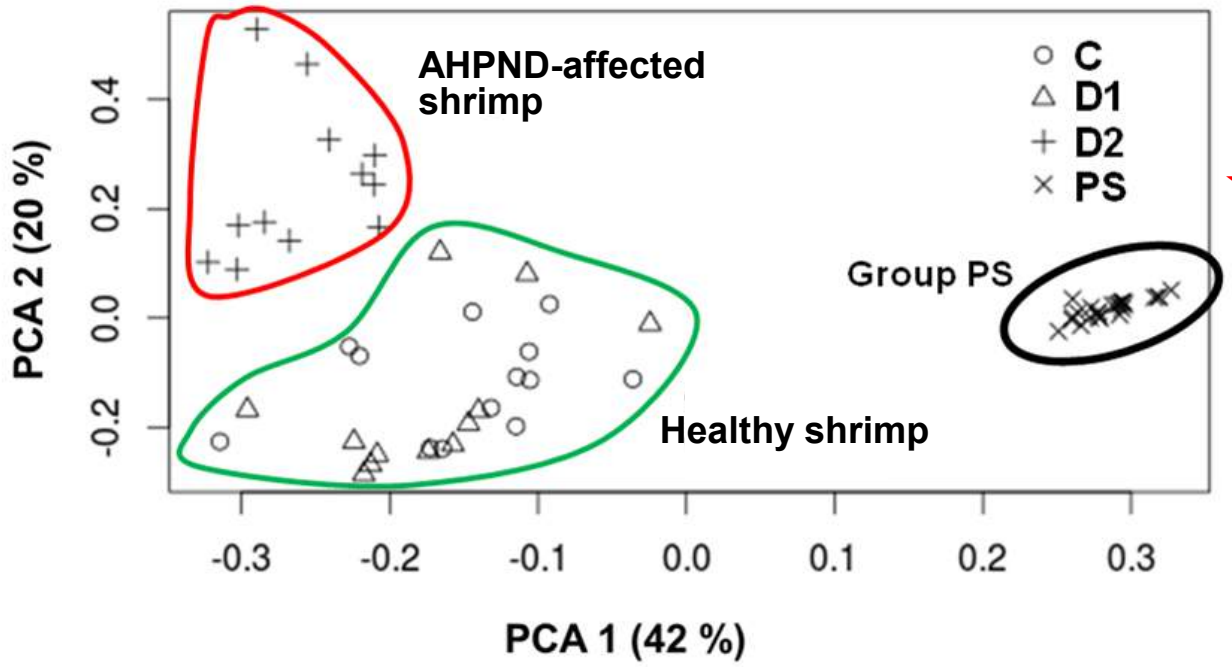




Shrimp size:~3 cm



Microbiome similarity

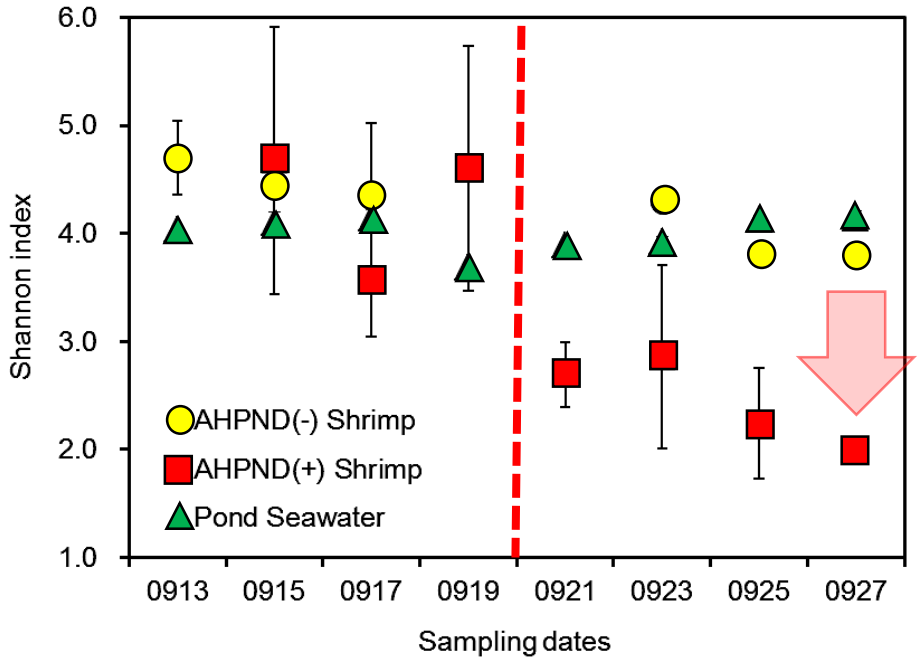


★Pond seawater samples were distinctly separated from the bacterial communities of shrimp

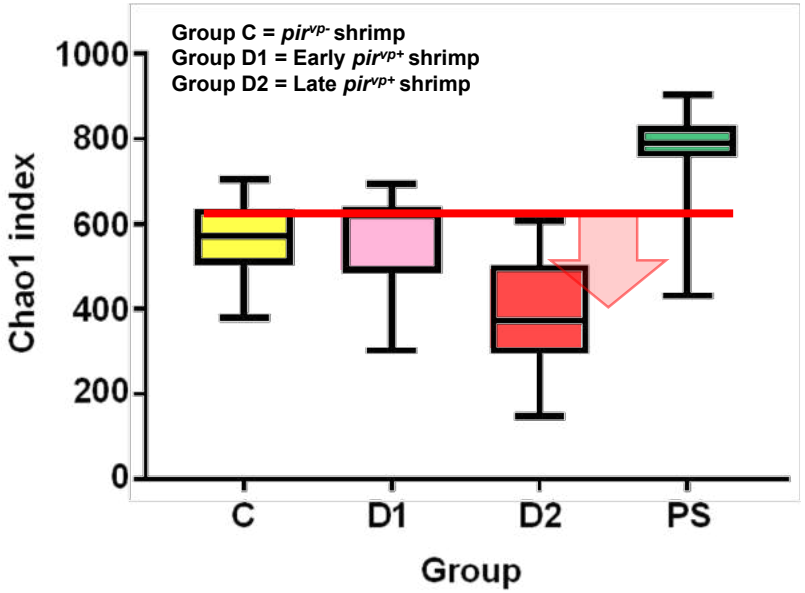
- **Low correction between the microbiota of shrimp stomach and pond seawater**
- **Microbiota profiles between AHPND-affected and healthy shrimp were different**

Microbial diversity in shrimp and pond water

After water flood, microbial diversity suddenly shifted in *pir*$^{vp+}$ shrimp

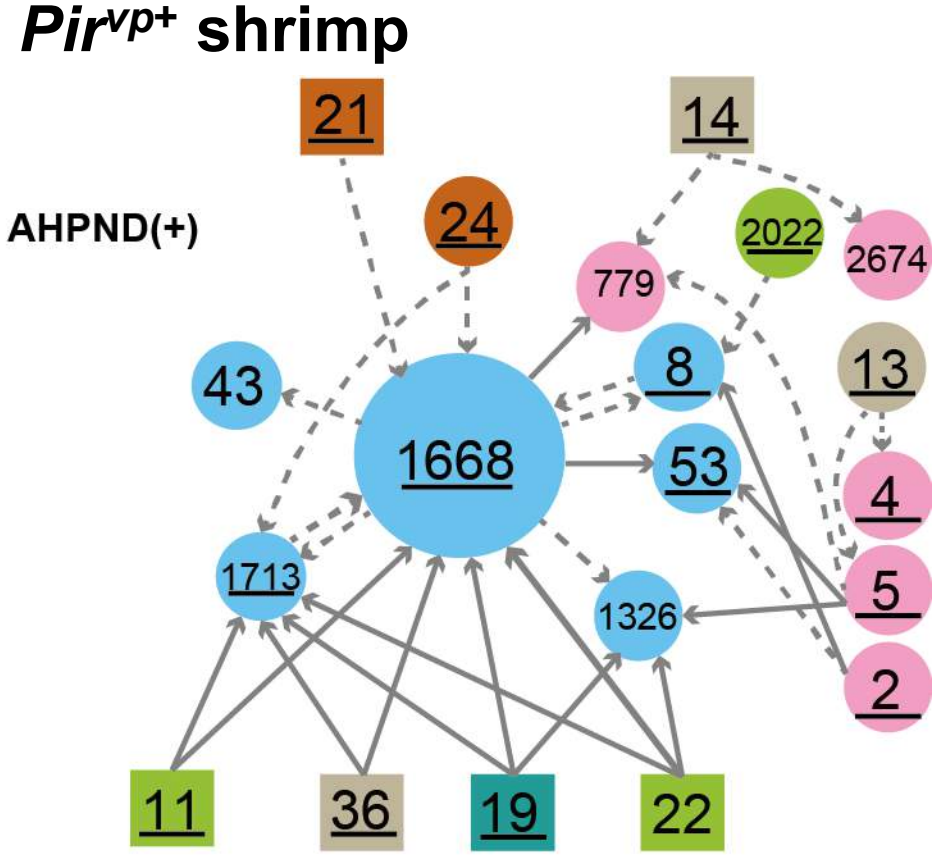


A lower bacterial richness in Group D2 shrimp



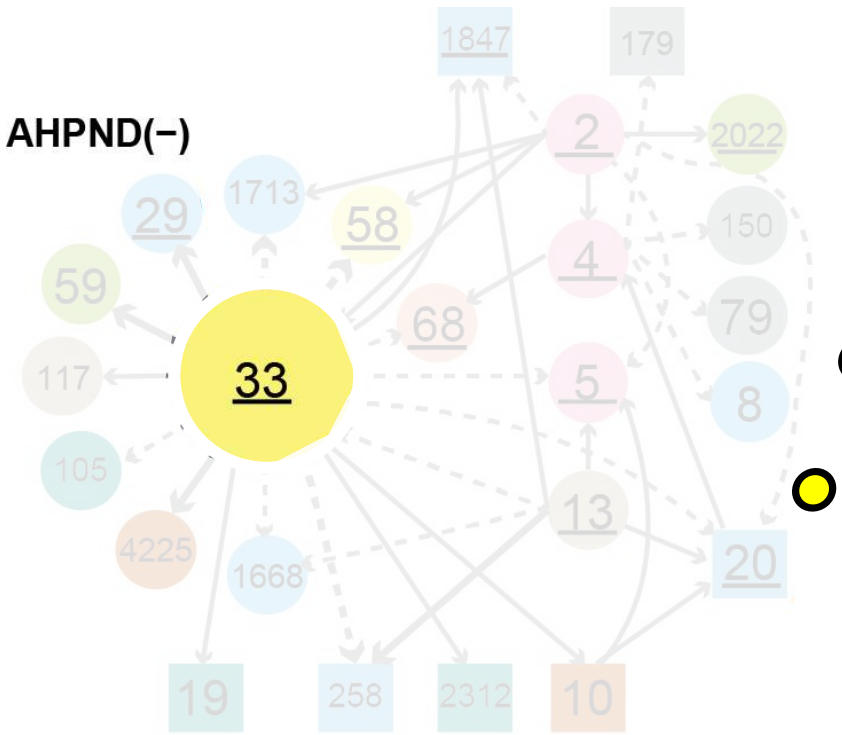
Bacterial species richness in some shrimp stomachs declined after heavy rain and during the development of AHPND Infection. Other shrimp stayed in healthy balance.

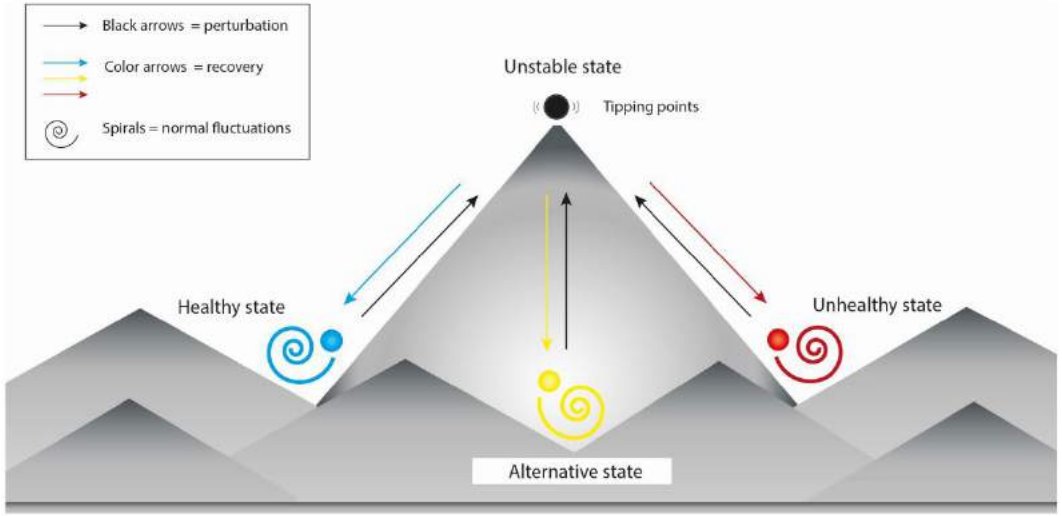
Vibrio-related consensus interactions in the *pir*$^{vp-}$ and *pir*$^{vp+}$ stomach communities



Important hubs
-- *Vibrio* OTUs (1668, 1713, and 8)

A potential probiotic to suppress the AHPND outbreak





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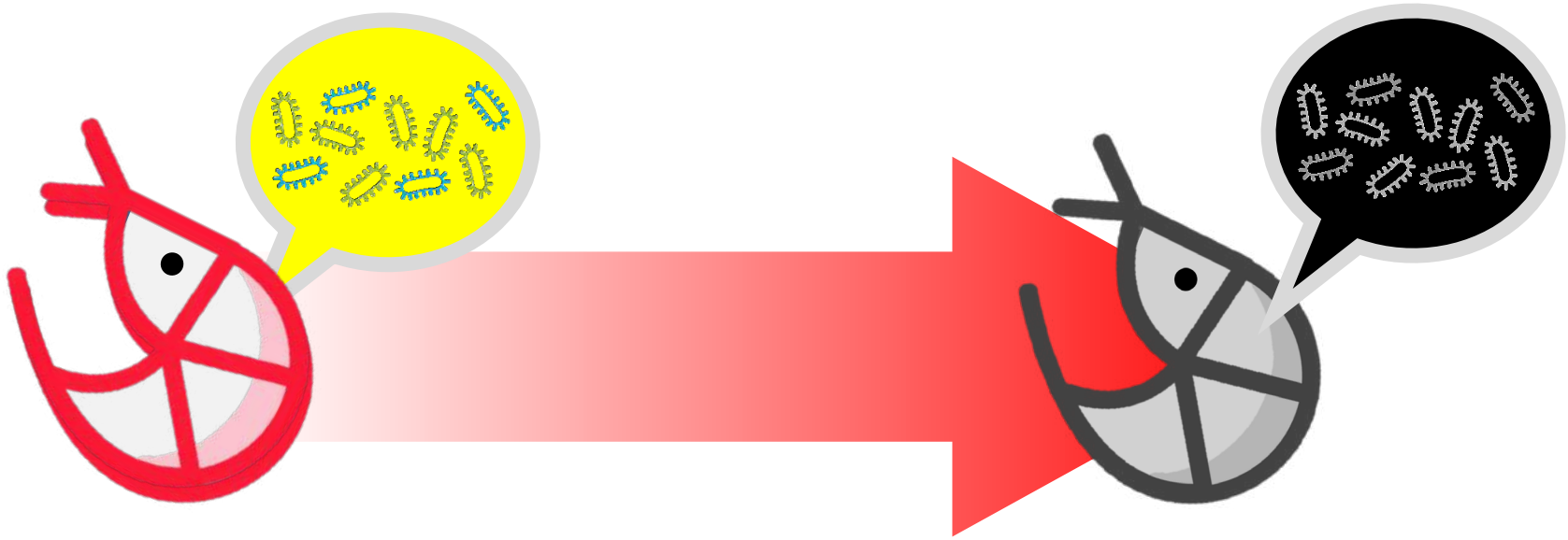
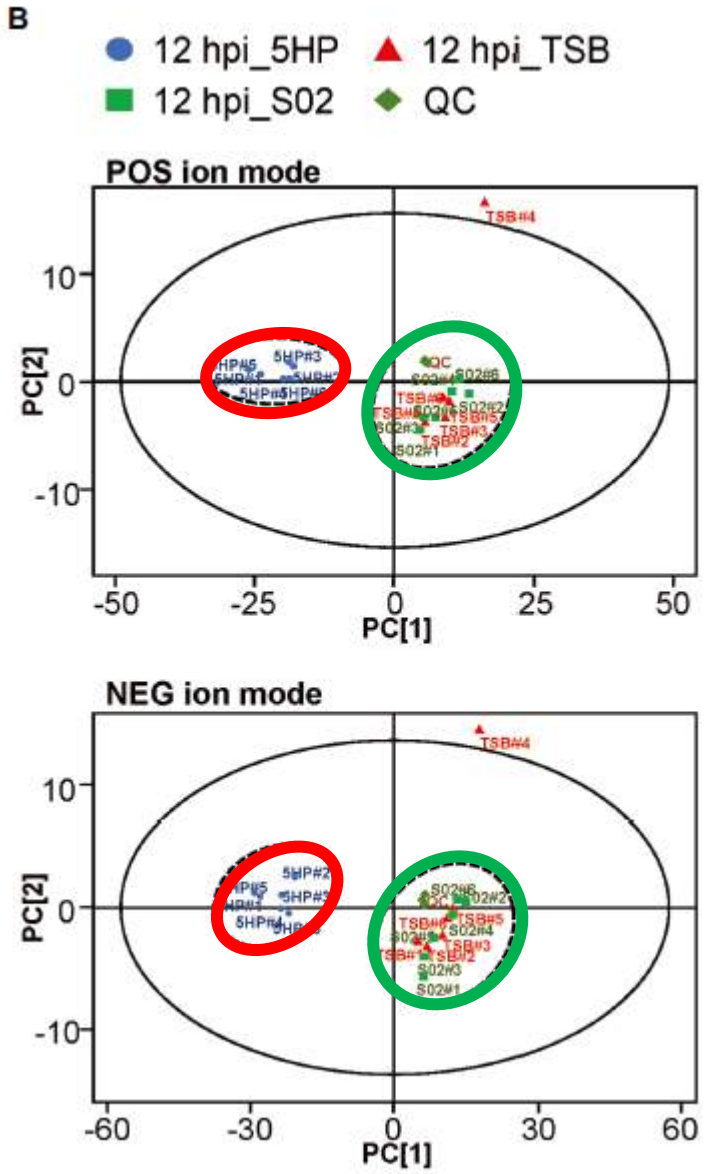
Dysbiosis in AHPND-affected shrimp

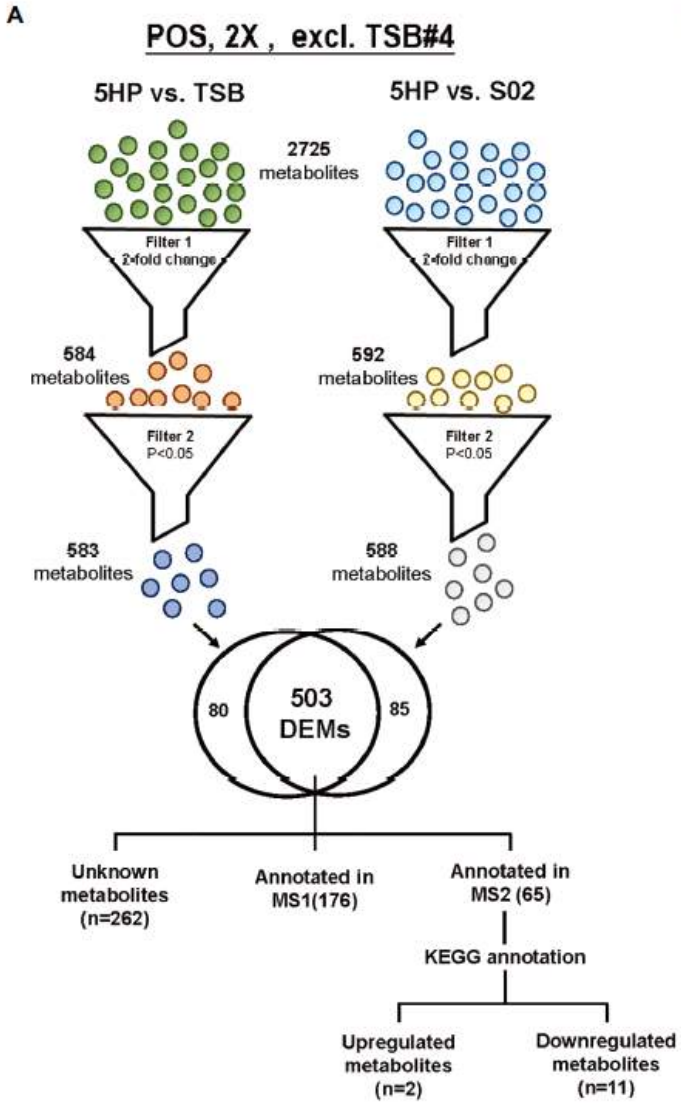


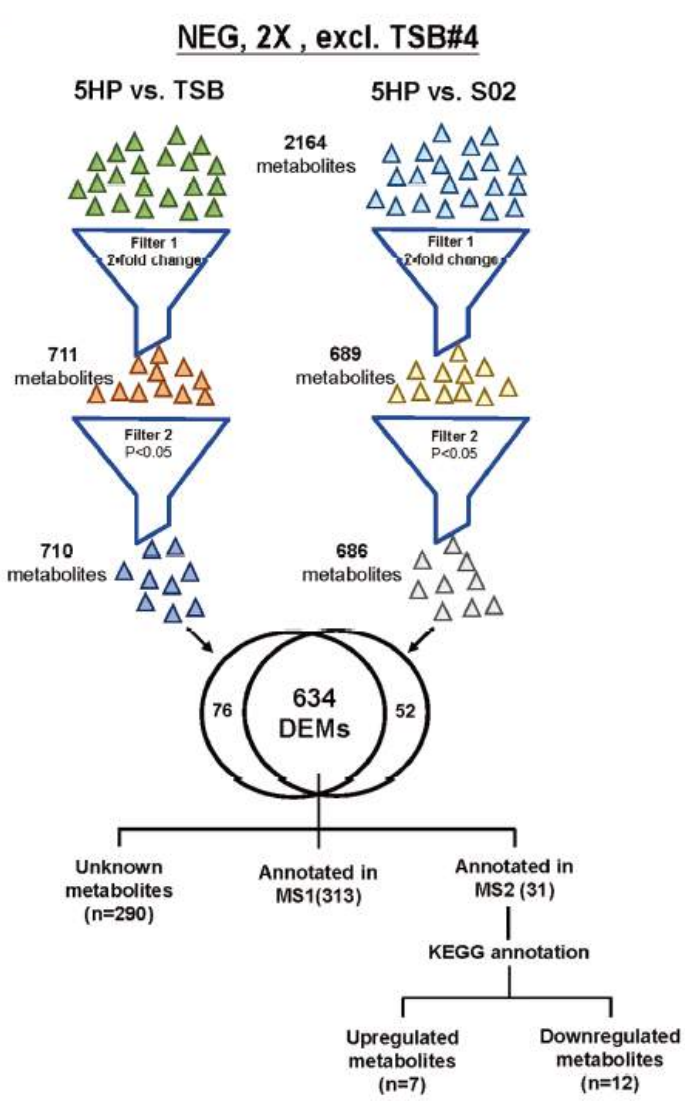
Bacteria metabolism in shrimp stomach

Host metabolism [shrimp stomach]

Metabolic change in AHPND-affected stomach

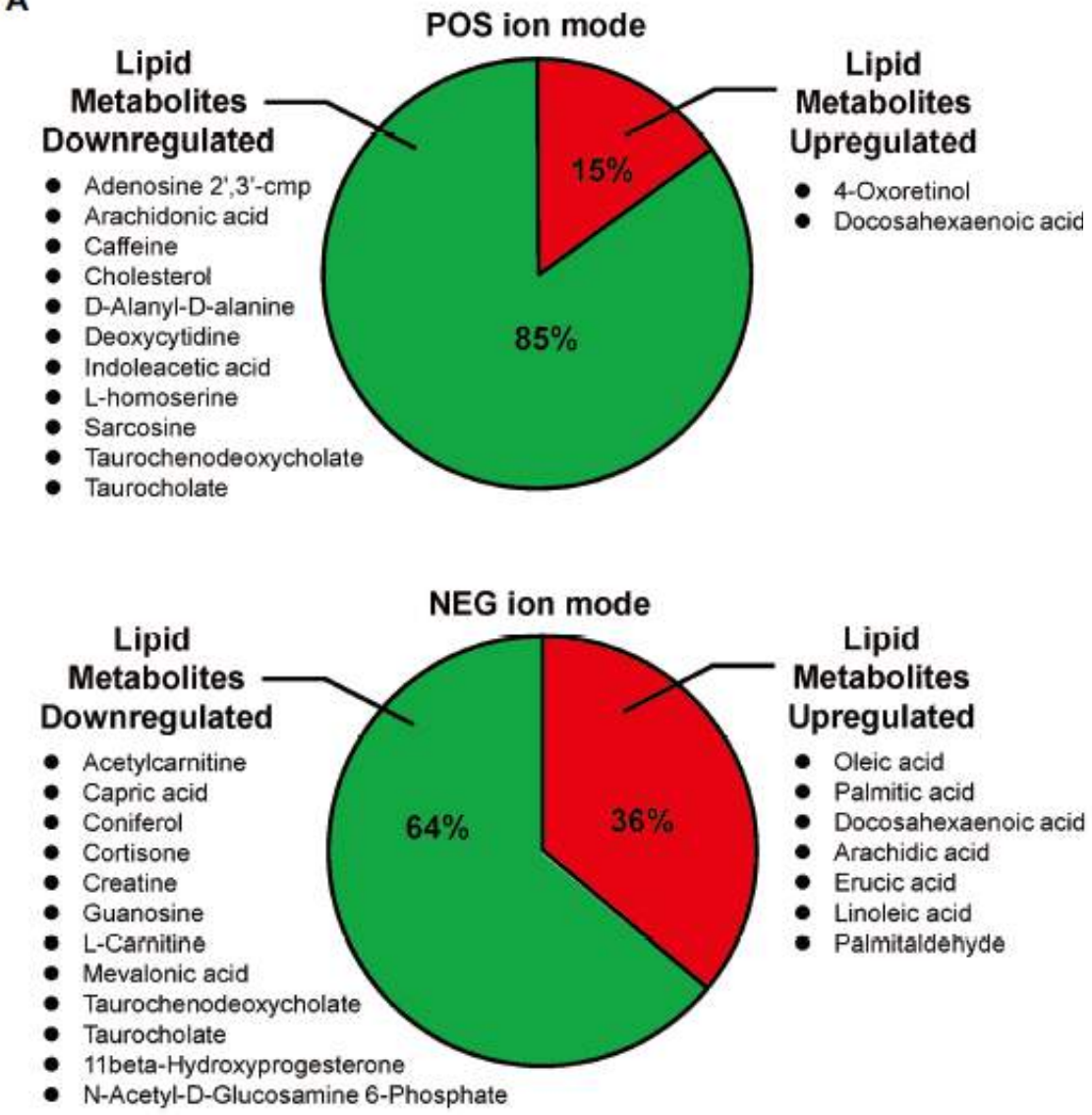




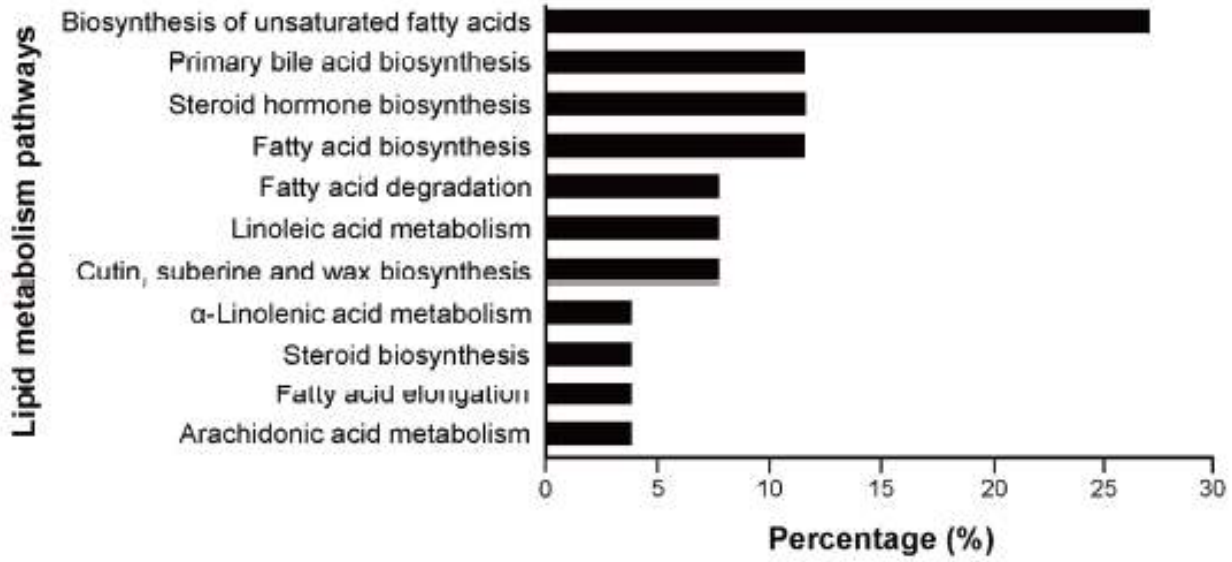


Metabolic change in AHPND-affected stomach

A



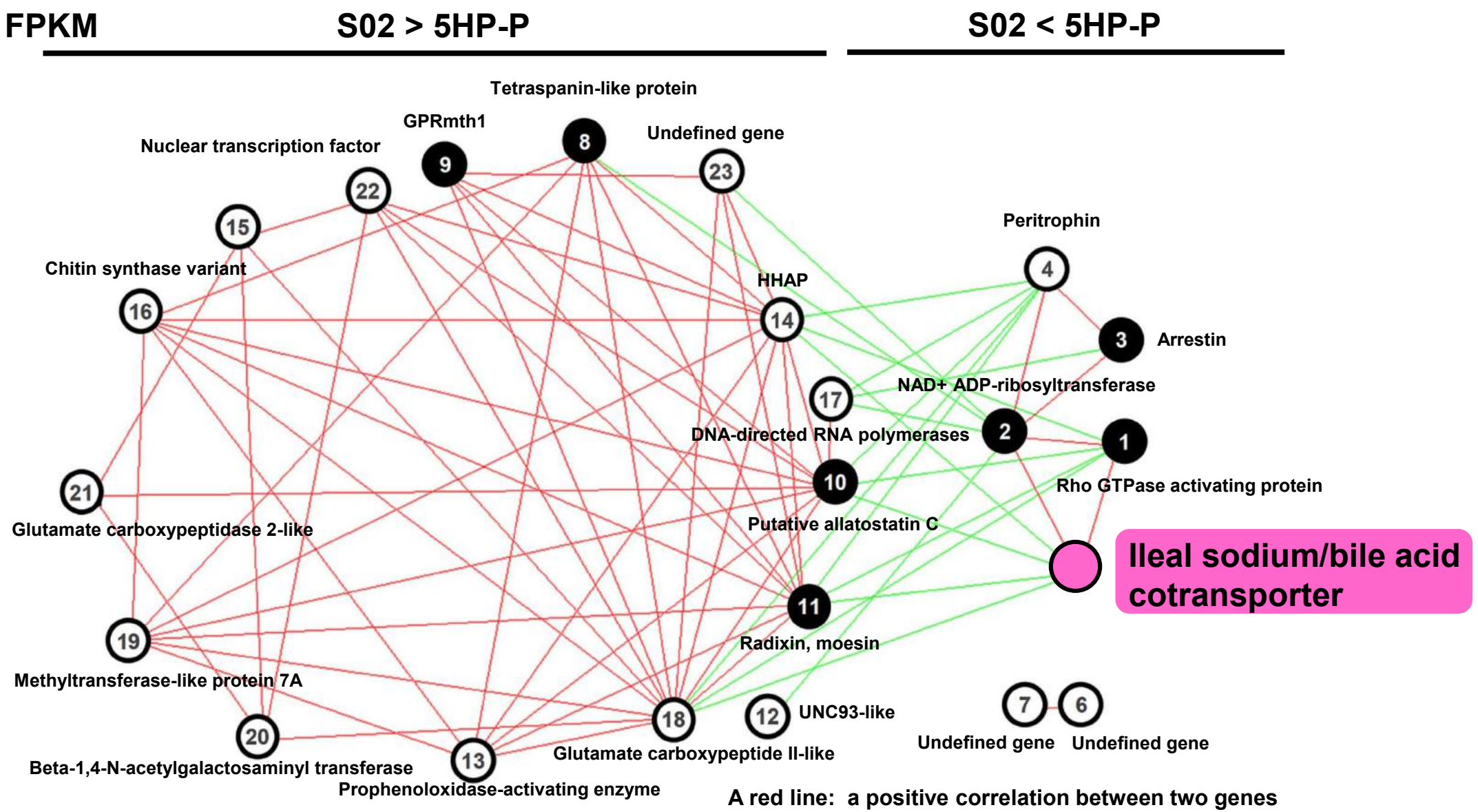
B



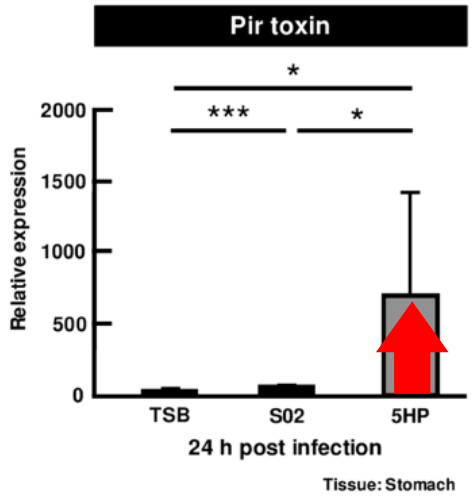
The top 3 changed metabolic pathways

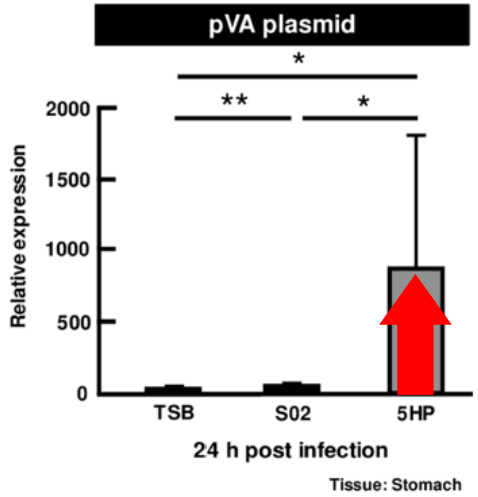
1. Biosynthesis of unsaturated fatty acids
2. Primary bile acid biosynthesis
3. Fatty acid biosynthesis

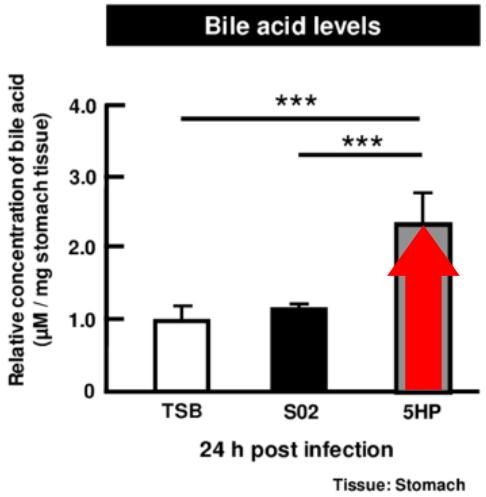
Gene-to-gene network of AHPND pathogenesis



AHPND pathogenesis is associated with increased bile acids

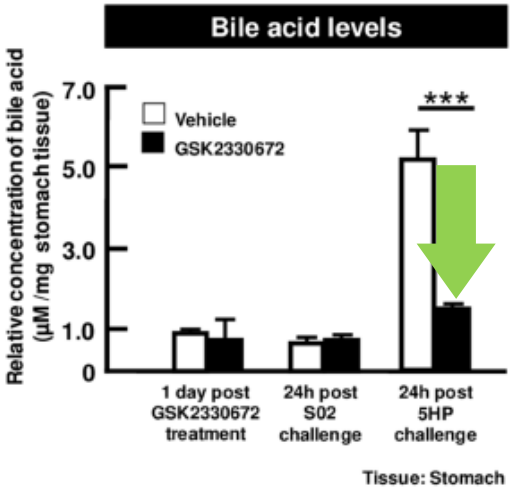


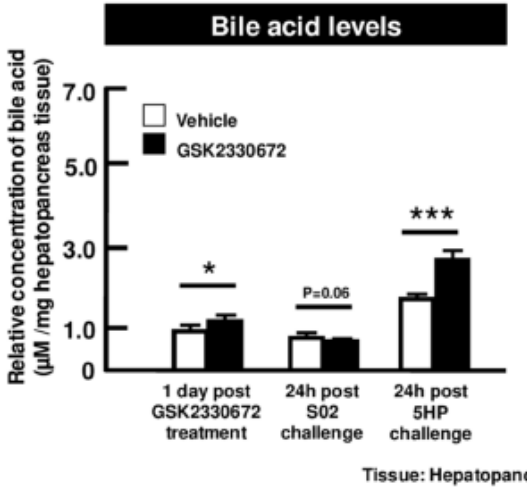


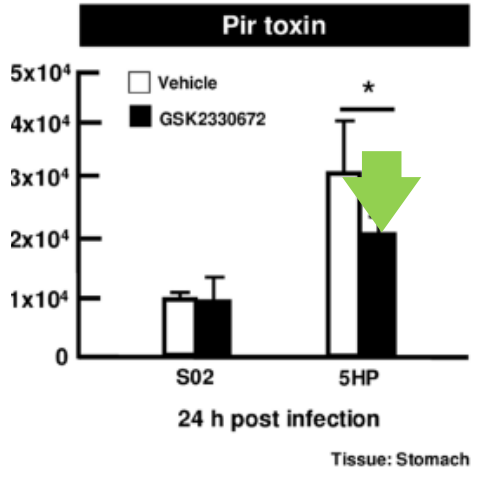


AHPND infection

Bile acid amount in stomach



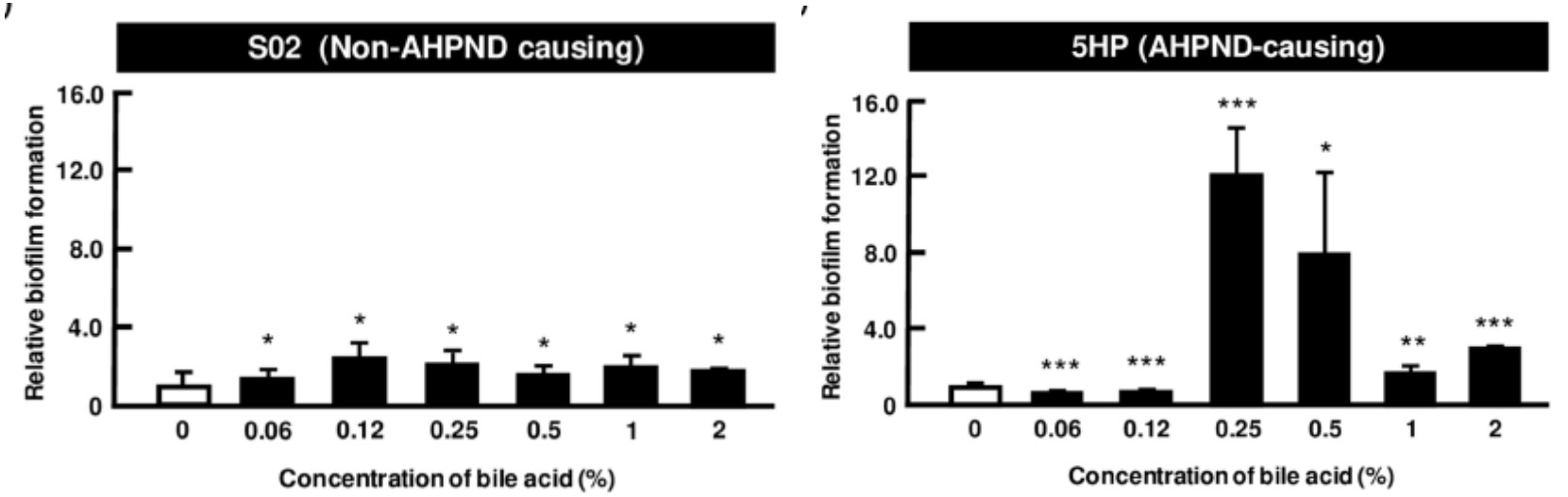




Lower bile acids

the number of AHPND-causing bacteria

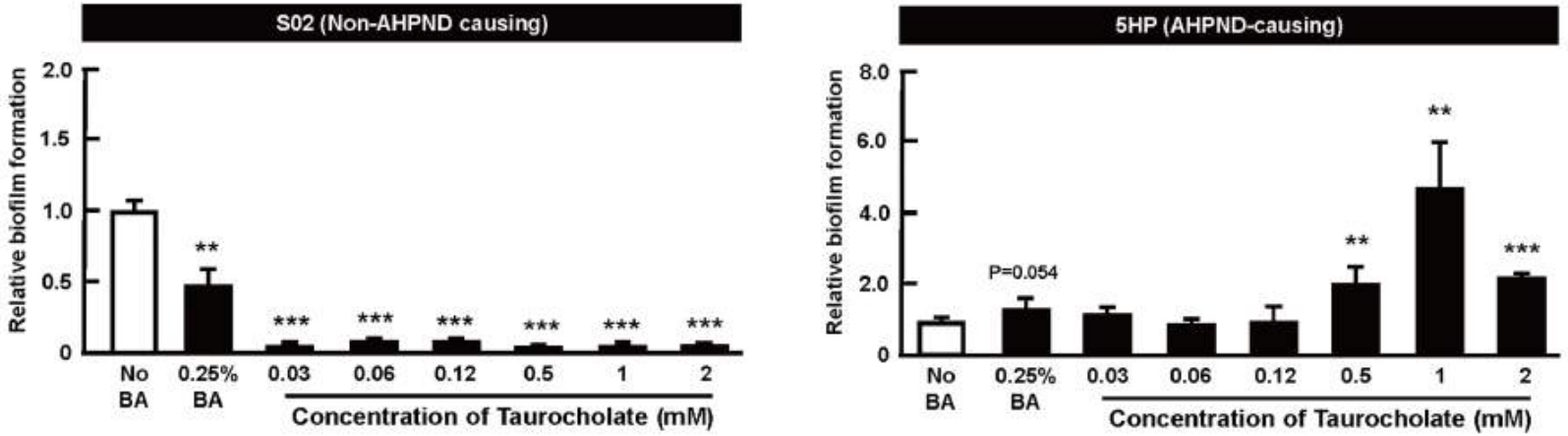
Bile acids induce biofilm formation in AHPND-causing *V. parahaemolyticus*



Bile acids

Biofilm formation

Primary bile acid metabolites – taurocholate (a taurine-conjugated form of the primary bile acid)

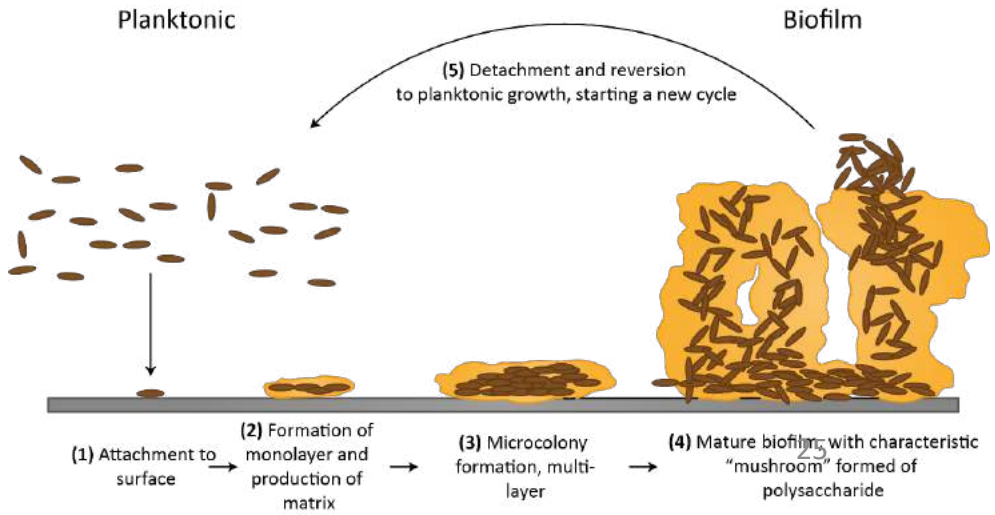


Taurocholate

Biofilm formation

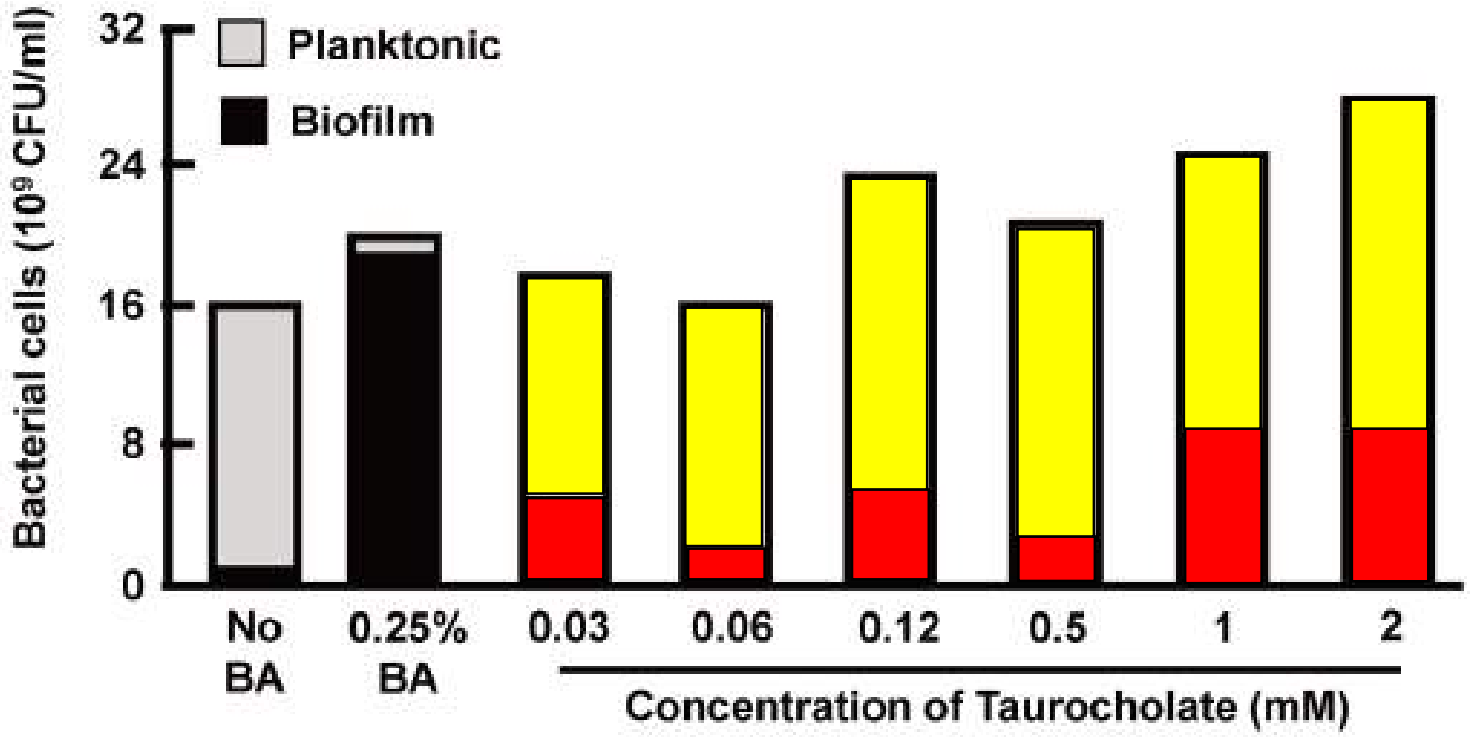
Bacterial biofilm in the gastrointestinal tract

- **What about biofilm**
  - microbial aggregates on surfaces
  - The biofilm lifestyle influences metabolic behaviour of the microbiota
  - Polymicrobial biofilms naturally grow throughout the gastrointestinal tract
    - both at the epithelial surface and in the lumen as mucin-attached and food particle-attached colonies.
- **Abnormal biofilm features**
  - associated with gastrointestinal diseases
  - characterization of biofilm alterations and cause-to-effect studies are warranted to elucidate their role in pathophysiology.



Koo et al., 2017; Motta et al., 2021

Taurocholate also increases the number of planktonic AHPND-causing *Vibrio parahaemolyticus*



Taurocholate

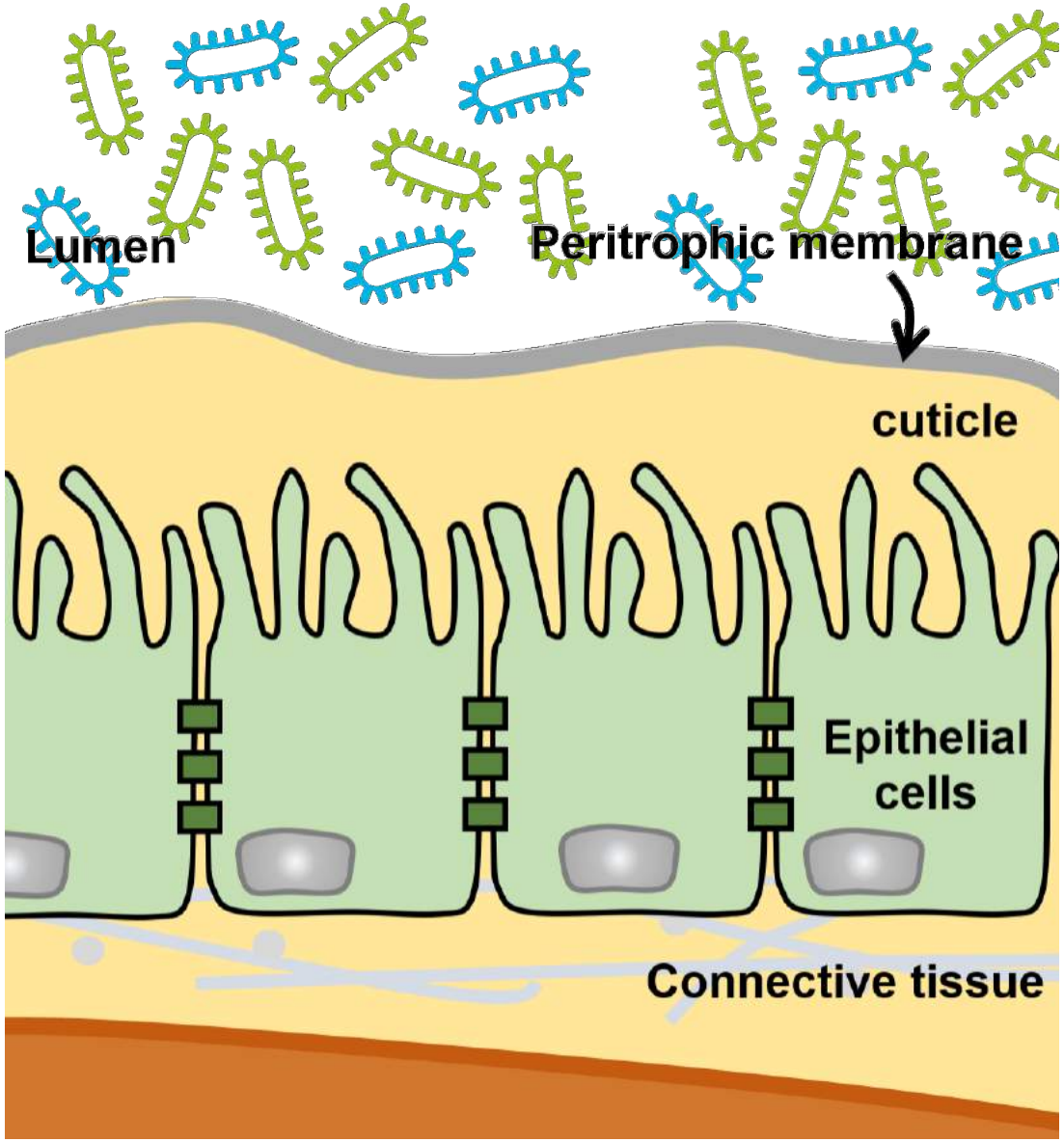
Biofilm formation

Planktonic Vibrio.

Higher concentrations of taurocholate

a positive effect on the number of planktonic AHPND-causing V. parahaemolyticus.

Shrimp digestive tract and diseases

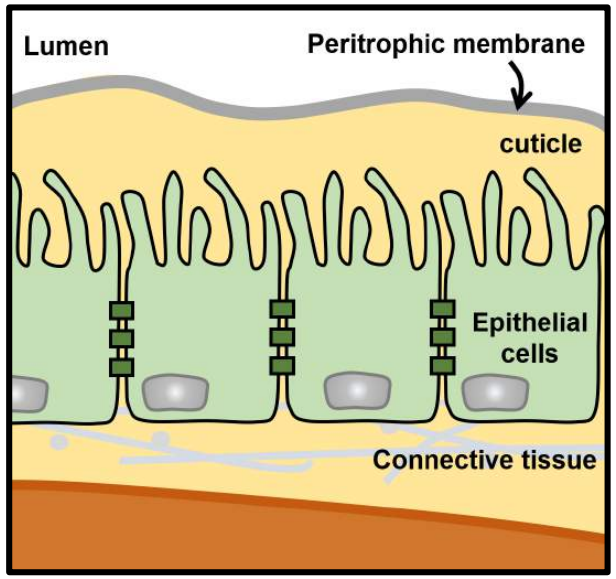


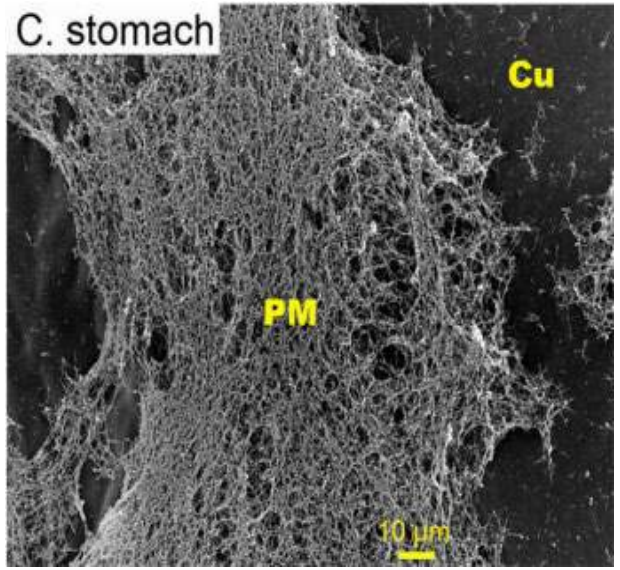
- **In case of AHPND**
  - **Microbiota**
  - **Stomach metabolism**
  - **Biofilm formation**
  - Peritrophic membrane
  - Cuticle
  - Epithelial cells
  - Hepatopancreas

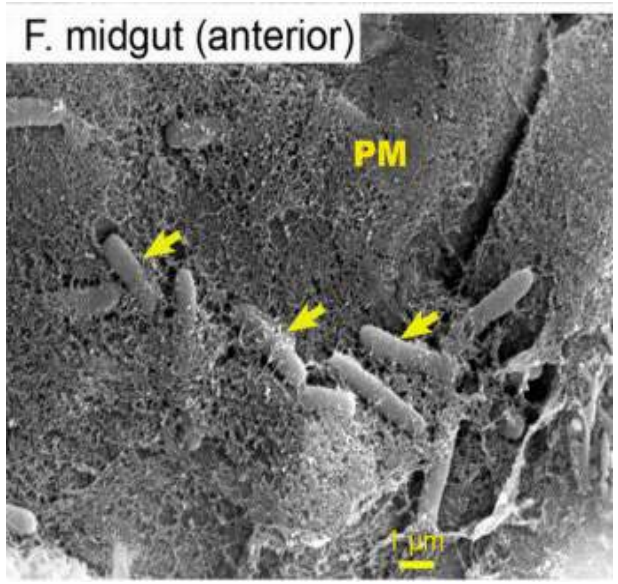
Vibrio colonization, distribution and migration

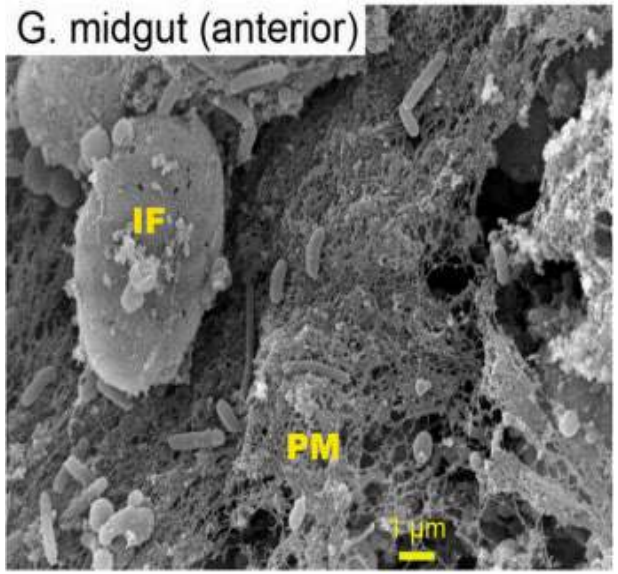
Peritrophic membrane and Cuticle layer

Presence of a normal flora in the GI tract of pond-cultured *Penaeus monodon*





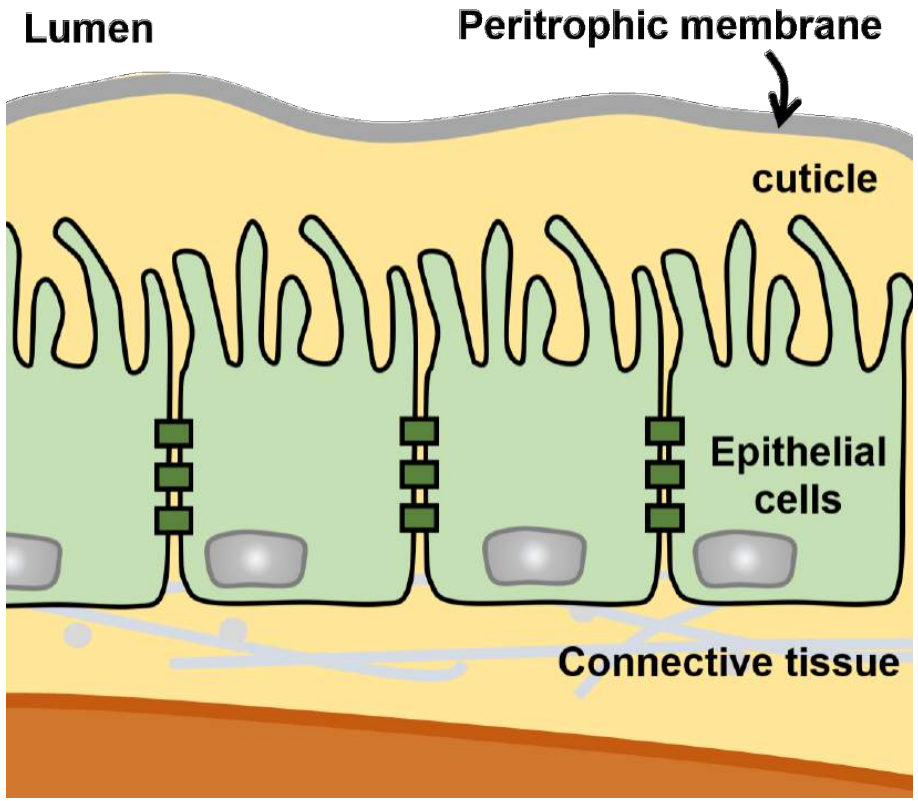




Peritrophic membrane (PM), cuticle (Cu), ingested food (IF) (Source: Soonthornchai *et al.*, 2015)

- **Peritrophic membrane**
  - chitin, proteoglycans, and protein [mainly peritrophin; PT]
  - Protects the epithelium from ingested bacteria
- **Cuticle layer**
  - a physical barrier to an invading pathogen
  - but also important as a mediator of bacterial colonization

The role of shrimp stomach structure in AHPND pathogenesis

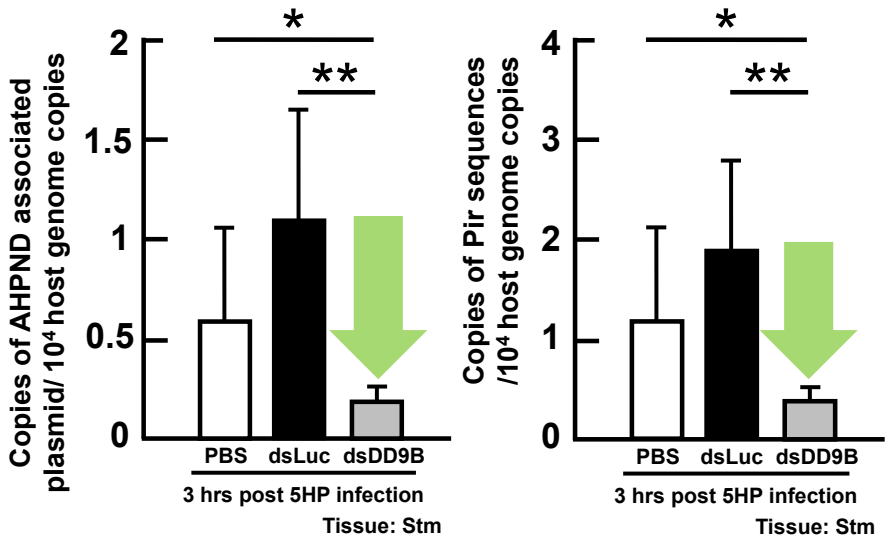


- Peritrophic membrane
  - Peritrophin (PT)
- Cuticle layer
  - DD9A/B

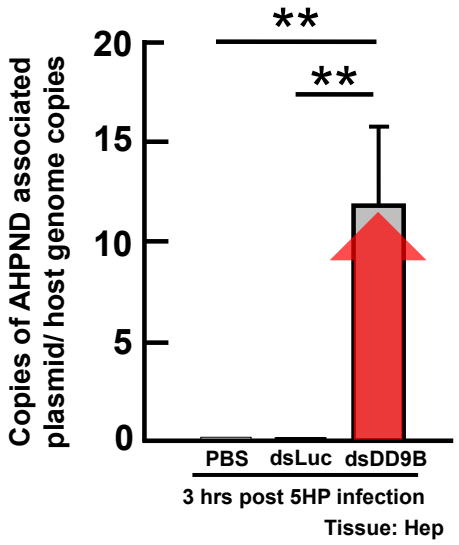
The involvement of gut structure in the colonization and the migration of AHPND-causing bacteria

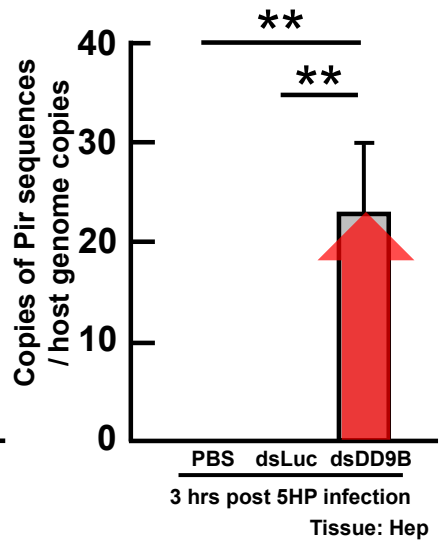
- Cuticle layer: DD9A/B

- Stomach

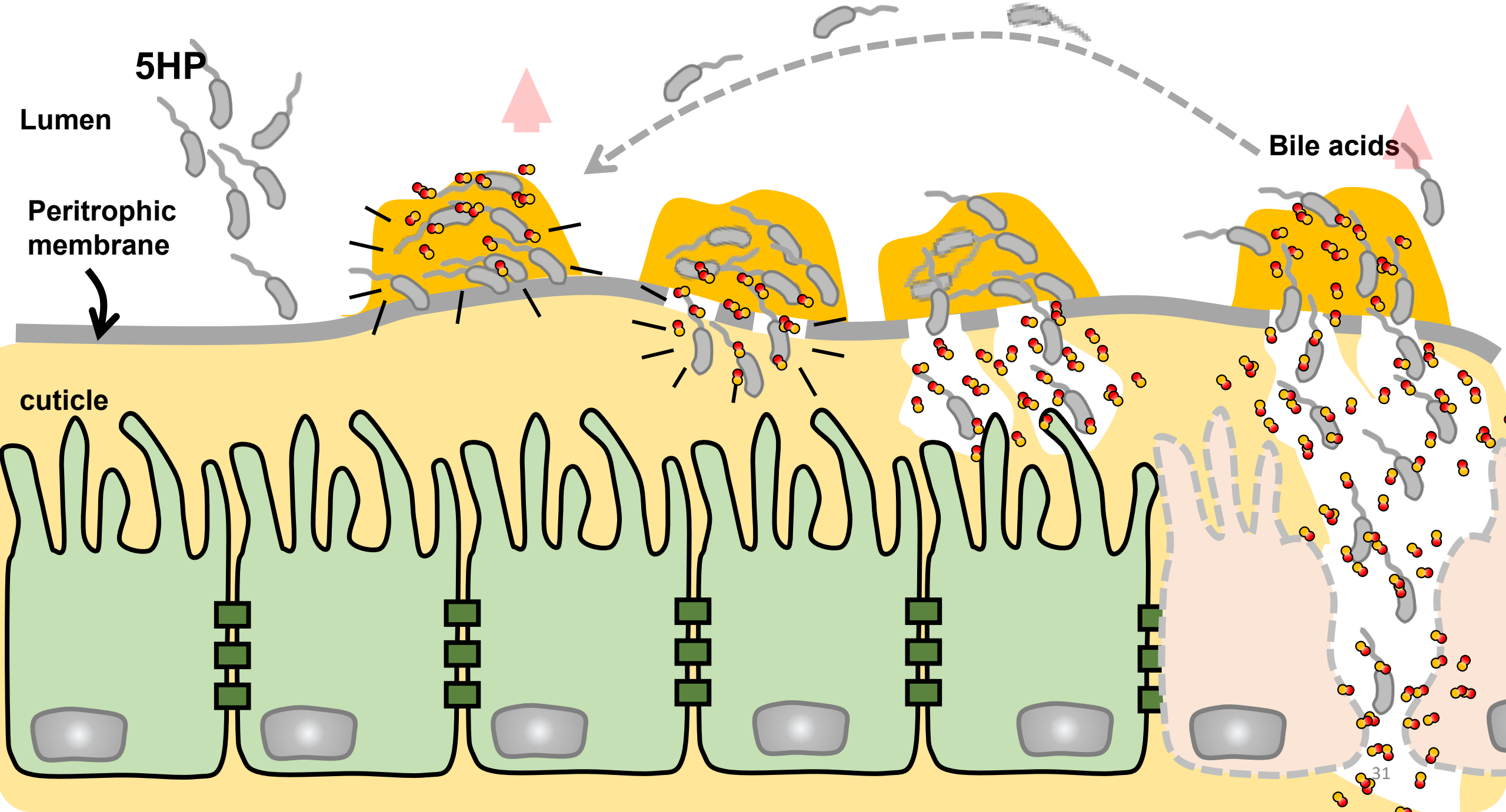


- Hepatopancreas





When cuticle layer is disrupted,
it facilitates the migration of AHPND-causing bacteria from stomach to hepatopancreases


5HP
Lumen
Peritrophic membrane
cuticle
Bile acids

Back to aquaculture and raising healthy shrimp

- **Breeding program**

Genetics shape the gut microbiome

- **The use of probiotics**

A practical way to promote animal health and prevent disease

- **Shrimp feed to promote shrimp gut health**

Reduce the risk of disease outbreak

Our publications

Microbiota 2017

Rho pathway 2018

Bile acids 2020

AHPND Review 2020

Taurocholate 2021

DD9A/B 2021

Aquaculture 521 (2020) 734990

Contents lists available at ScienceDirect

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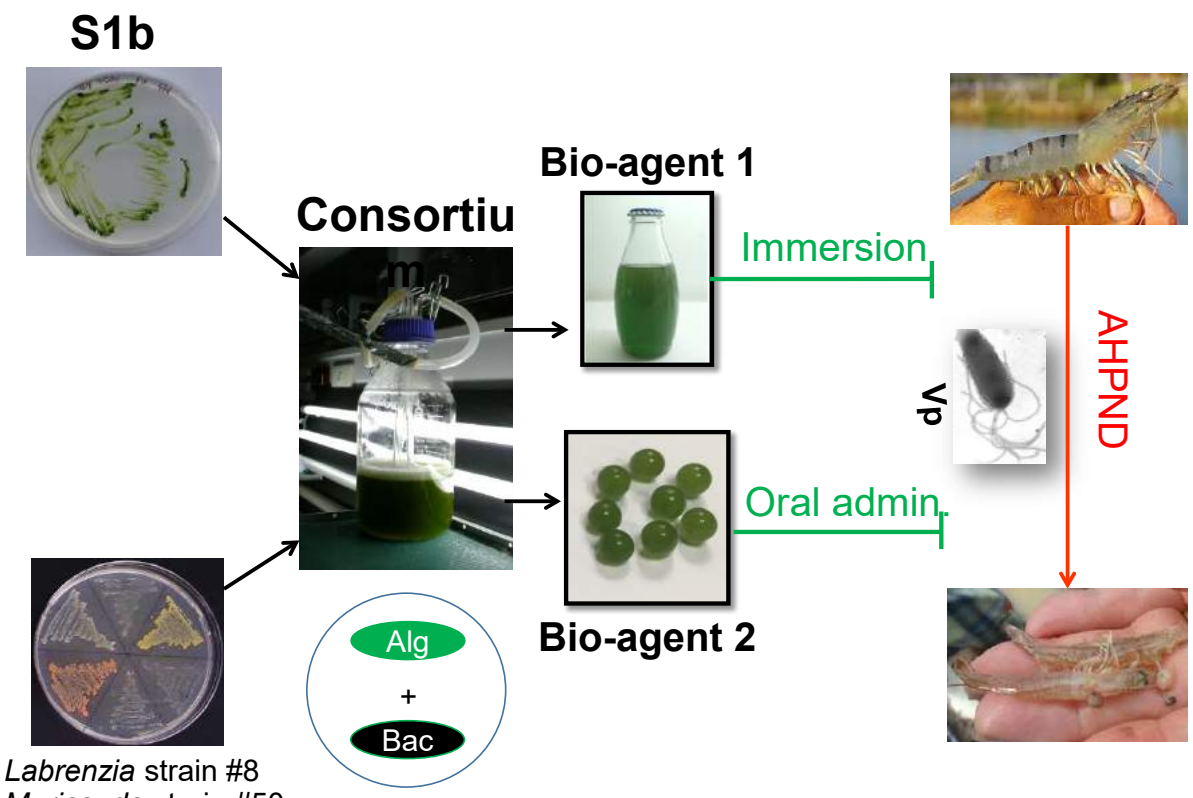
journal homepage: www.elsevier.com/locate/aquaculture

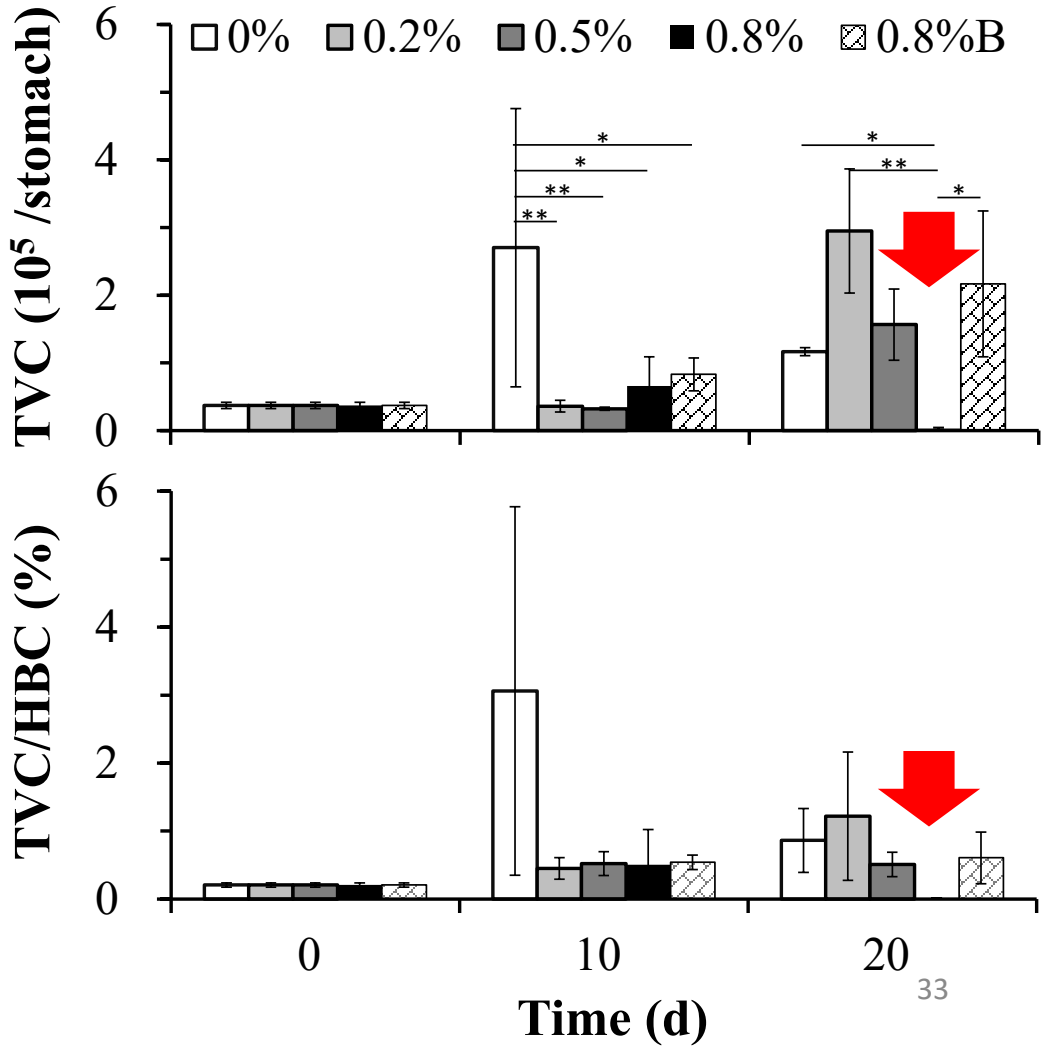
Biocontrol of acute hepatopancreatic necrosis disease (AHPND) in shrimp using a microalgal-bacterial consortium

Yu-Han Chang, Wan-Ching Kuo, Han-Chin Wang, Yi-Min Chen*

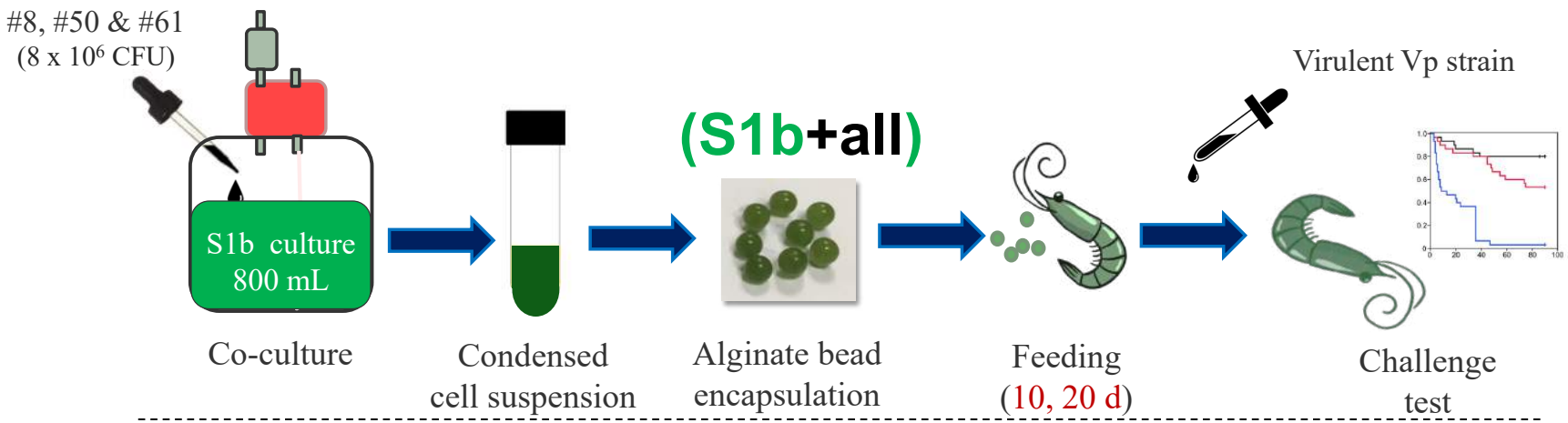
Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan 701, Taiwan

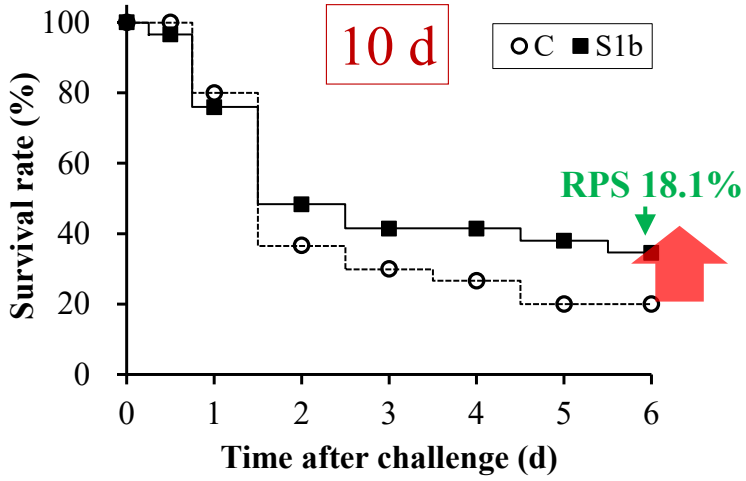


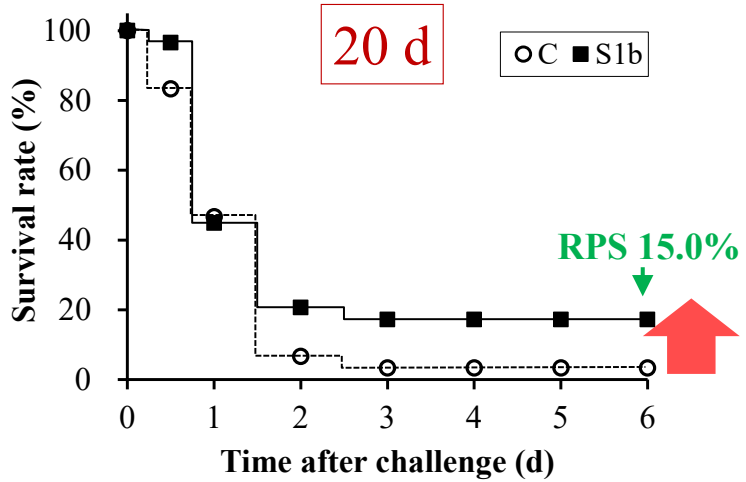
Changes in microbial communities in stomachs of whiteleg shrimp after shrimp had been fed 0~0.8% of their mean weight in alginate beads containing S1b+all every day for 10 days or 20 days.



Does the oral administration of 0.8% S1b+all encapsulated with co-culture prevent Vp infection?

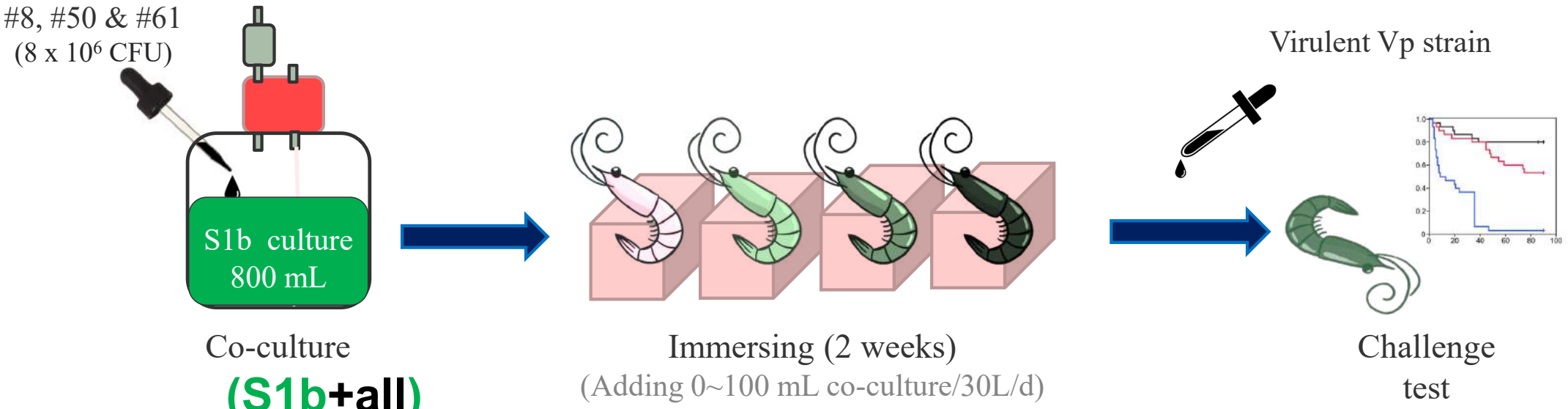


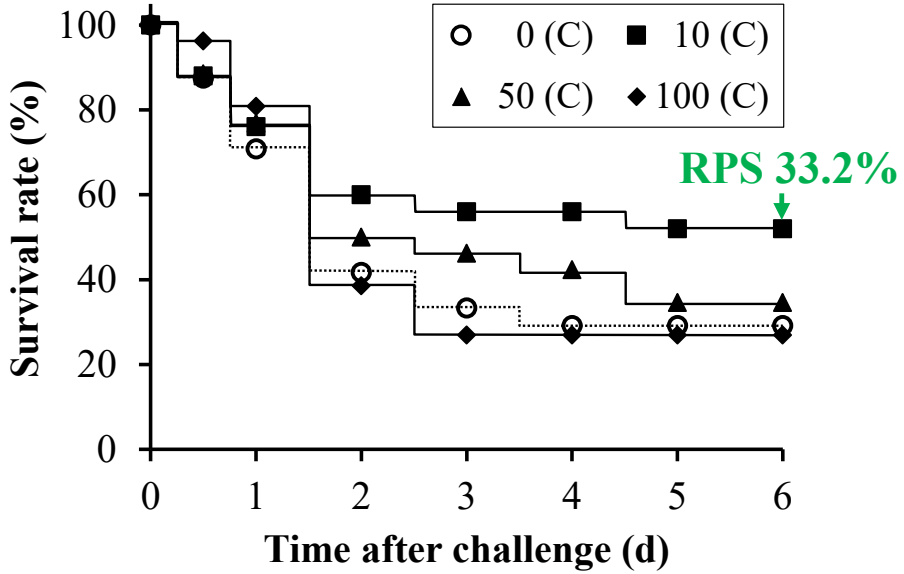




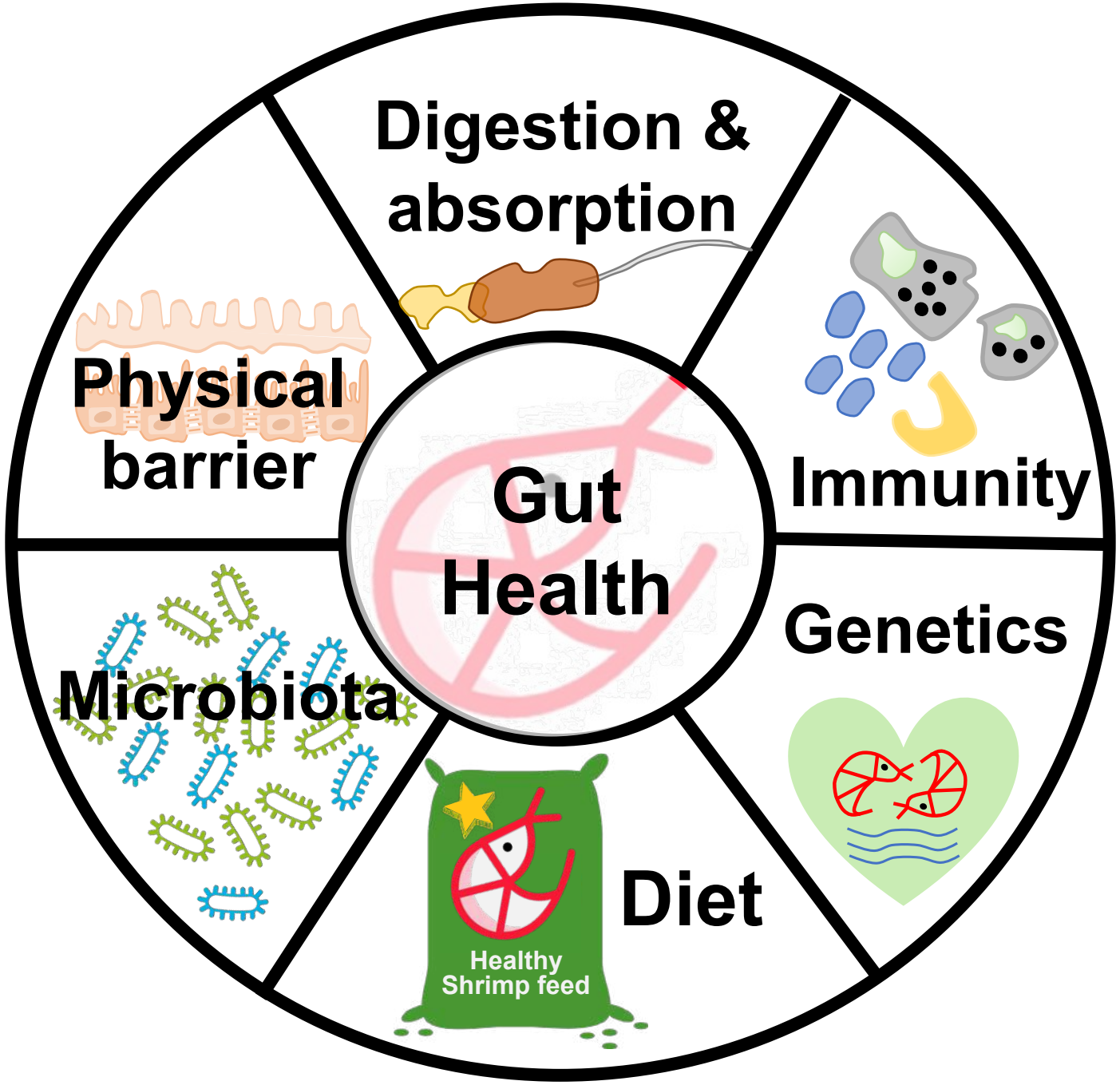
Feeding shrimp with 0.8% alginate bead for 10 or 20 day provides similar protection against Vp infection

Does the immersion also prevent Vp infection?





Applying a small dose of the S1b+all (10 mL/30L tank water) every day offered the optimal protection against Vp infection.


Digestion & absorption
Physical barrier
Immunity
Gut Health
Genetics
Microbiota
Diet
Healthy Shrimp feed

International Center for the Scientific Development of Shrimp Aquaculture

1931

國立成功大學

ICDSA

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前瞻蝦類養殖國際研發中心
International Center for the Scientific
Development of Shrimp Aquaculture

Director：Prof. Han-Ching Wang

Principal Investigator：Prof. Chu-Fang Lo

Nucleus Breeding Center (NBC) at Tainan

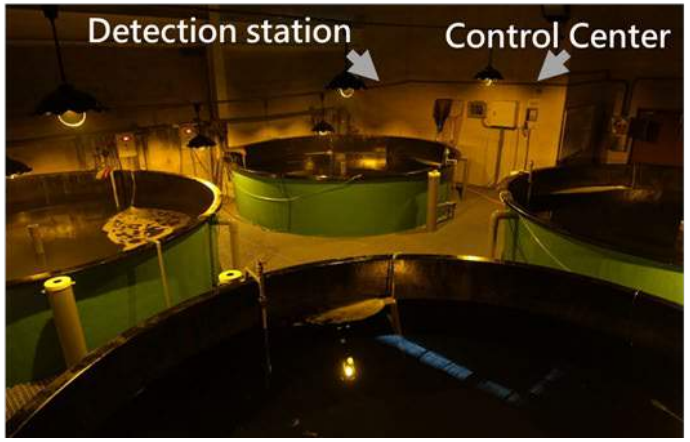
A shrimp multiplication center at Hualien

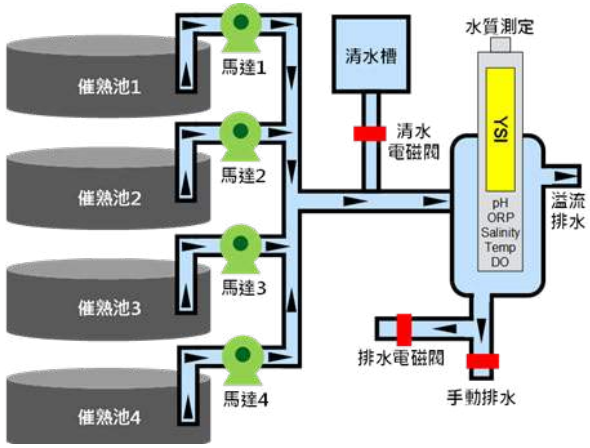
OIE reference laboratory for WSD/AHPND

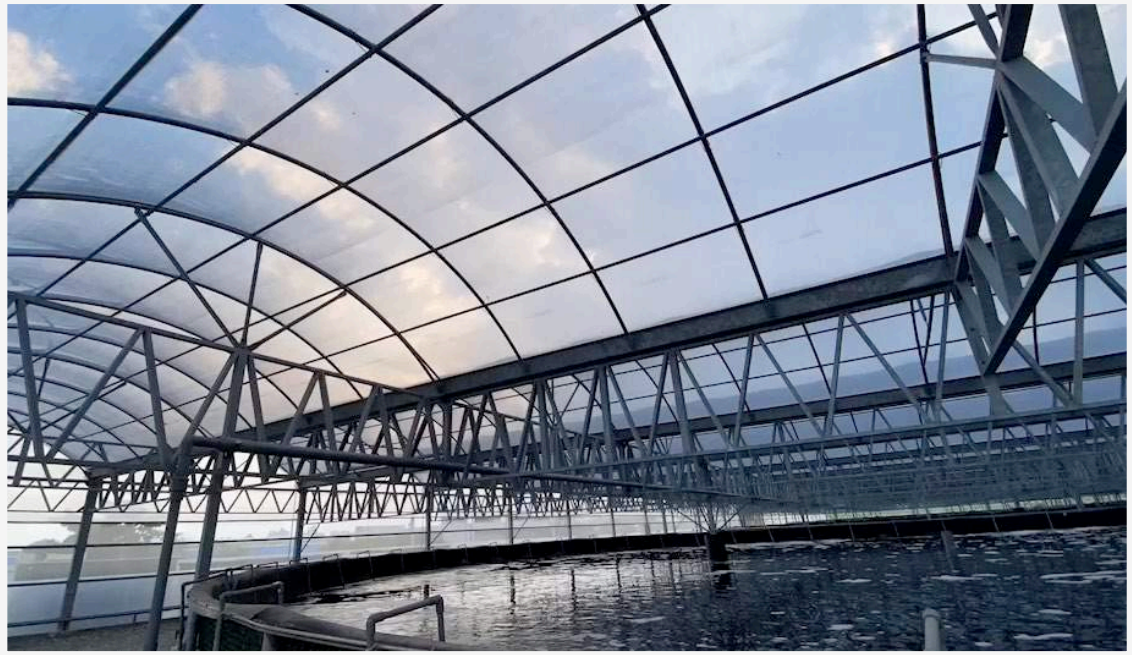
- ISO 17025 certified Lab
- WSSV PCR detection
- AHPND PCR detection

世界動物衛生組織蝦白點病參考實驗室
OIE Reference Laboratory for White Spot Disease
世界動物衛生組織蝦急性肝胰腺壞死病參考實驗室
OIE Reference Laboratory for Acute Hepatopancreatic Necrosis Disease

Breakthrough of the technology








450噸HDPE養殖池
（共12池）
450噸HDPE養
殖池（共2池）
600噸儲水池
（共4池)
50噸FRP養殖池
（共6池）

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SGS

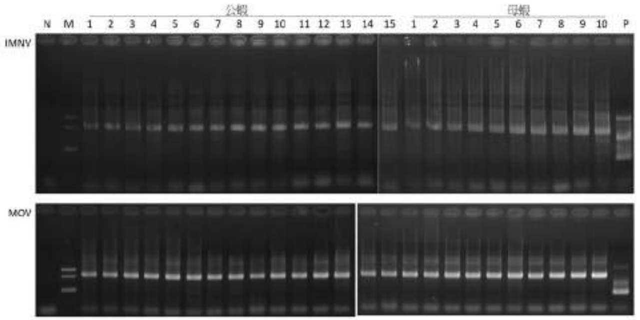
食品實驗室-高雄
FOOD LAB-KAOHSIUNG
測試報告
Test Report

國立成功大學-前瞻蝦類養殖國際研發中心
台南市安南區安明路三段500號

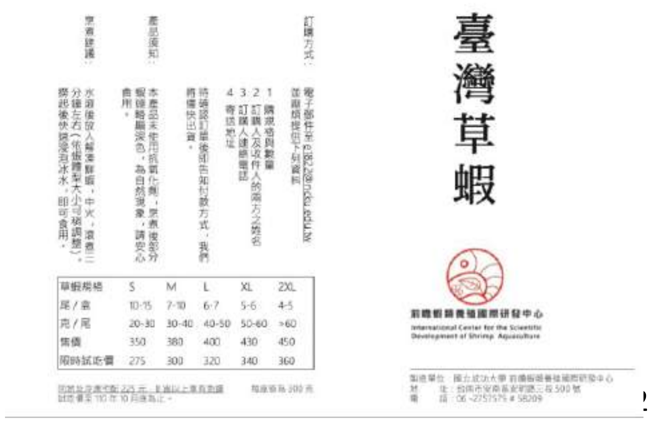
報告編號：AVA21803963V
報告日期：2021/06/31

測試項目	測試方法	測試結果	定量/偵測極限(註3)	單位
★ 動物用藥多重殘留分析(二)(48品項)	---	---	---	---
★ 西氟沙星	108年10月8日衛生福利部衛授食字第1081901669號公告修正食品中動物用藥殘留量檢驗方法－多重殘留分析(二)，以液相層析串聯式質譜儀(LC/MS/MS)檢測。	未檢出	0.01	ppm(mg/kg)
★ 恩氟喹啉羧酸		未檢出	0.01	ppm(mg/kg)
★ 大安氟喹啉羧酸		未檢出	0.01	ppm(mg/kg)
★ 二氟喹啉羧酸		未檢出	0.01	ppm(mg/kg)
★ Fleroxacin		未檢出	0.01	ppm(mg/kg)
★ 氟滅菌		未檢出	0.01	ppm(mg/kg)
★ Lomefloxacin		未檢出	0.01	ppm(mg/kg)
★ Marbofloxacin		未檢出	0.01	ppm(mg/kg)
★ 那利得酸		未檢出	0.01	ppm(mg/kg)
★ 諾氟喹啉羧酸		未檢出	0.01	ppm(mg/kg)
★ 歐索林酸		未檢出	0.01	ppm(mg/kg)
★ Pefloxacin		未檢出	0.01	ppm(mg/kg)
★ Pipemidic acid		未檢出	0.01	ppm(mg/kg)
★ Piromidic acid		未檢出	0.01	ppm(mg/kg)
★ 沙拉氟喹啉羧酸				
★ Azaperol				
★ Azaperone				
★ Carazolol				
★ 氯吡啶				
★ Dicyclanil				
★ Eprinomectin				
★ 衣索巴				
★ Fluazuron				
★ 摩朗得				
★ 歐美德普				
★ Tetramisole				
★ 三氯仿				
★ 三甲氧苄氨嘧啶				
★ Succinylsulfathiazole				
★ Sulfabenzamide				
★ 乙醯磺胺				
★ 磺胺氯吡嗪				
★ 磺胺嘧啶				

本商品通過多項SGS檢驗合格
- ✓ 抗生素殘留測試
- ✓ 重金屬測試（無機砷、甲基汞 鉛、鎘）
- ✓ 48項動物用藥殘留測試
- ✓ 孔雀綠與還原型孔雀綠測試
- ✓ 硝基呋喃代謝物測試



10 shrimp viruses free

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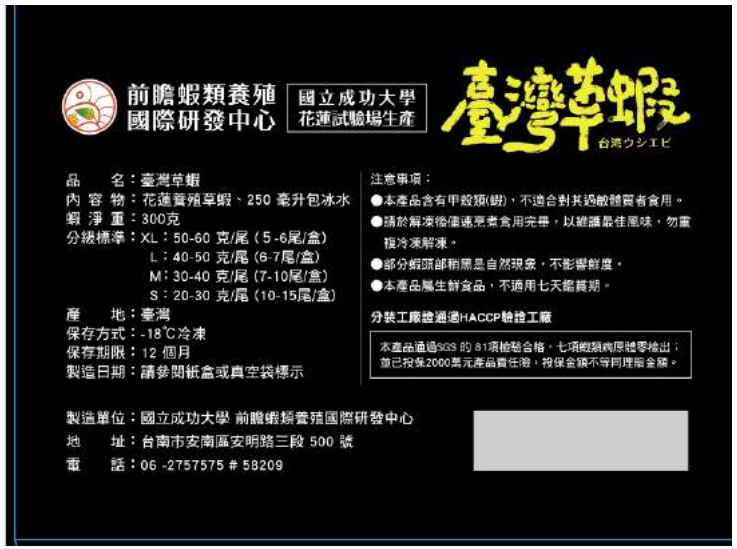
國際研發中心
國立成功大學
花蓮試驗場生產
臺灣草蝦
台湾ウシエビ
科學化無用藥養殖
高食品安全
■S ■M ■L ■XL
須冷凍
非供即食，應充分加熱。
淨重300公克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國際研發中心
國立成功大學
花蓮試驗場生產
臺灣草蝦
台湾ウシエビ
品　名：臺灣草蝦
內 容 物：花蓮養殖草蝦、250 毫升包冰水
蝦 淨 重：300克
分級標準：XL：50-60 克/尾（5-6尾/盒）
L：40-50 克/尾 (6-7尾/盒)
M：30-40 克/尾 (7-10尾/盒)
S：20-30 克/尾 (10-15尾/盒)
產　地：臺灣
保存方式：-18℃冷凍
保存期限：12 個月
製造日期：請參閱紙盒或真空袋標示
注意事項：
●本產品含有甲殼類(蝦)，不適合對其過敏體質者食用。
●請於解凍後儘速烹煮食用完畢，以維護最佳風味，勿重複冷凍解凍。
●部分蝦頭部稍黑是自然現象，不影響鮮度。
●本產品屬生鮮食品，不適用七天鑑賞期。
分裝工廠證通過HACCP驗證工廠
本產品通過SGS 的 81項檢驗合格，七項麩類病原體零檢出；並已投保2000萬元產品責任險，投保金額不等同理賠金額。
製造單位：國立成功大學 前瞻蝦類養殖國際研發中心
地　址：台南市安南區安明路三段 500 號
電　話：06 -2757575 # 58209


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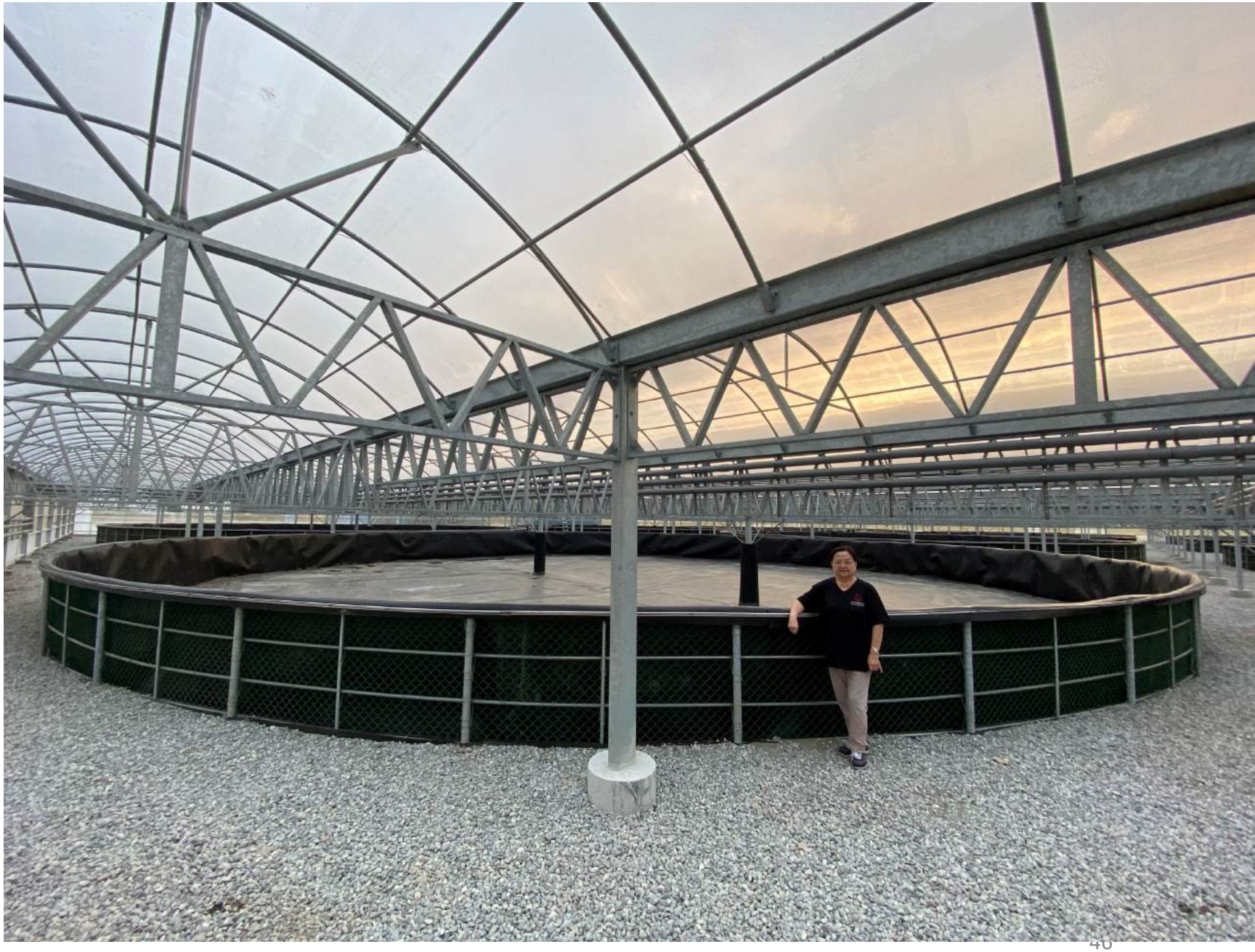

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International Center for the Scientific Development of Shrimp Aquaculture

REVIEWS IN Aquaculture

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2020

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2021