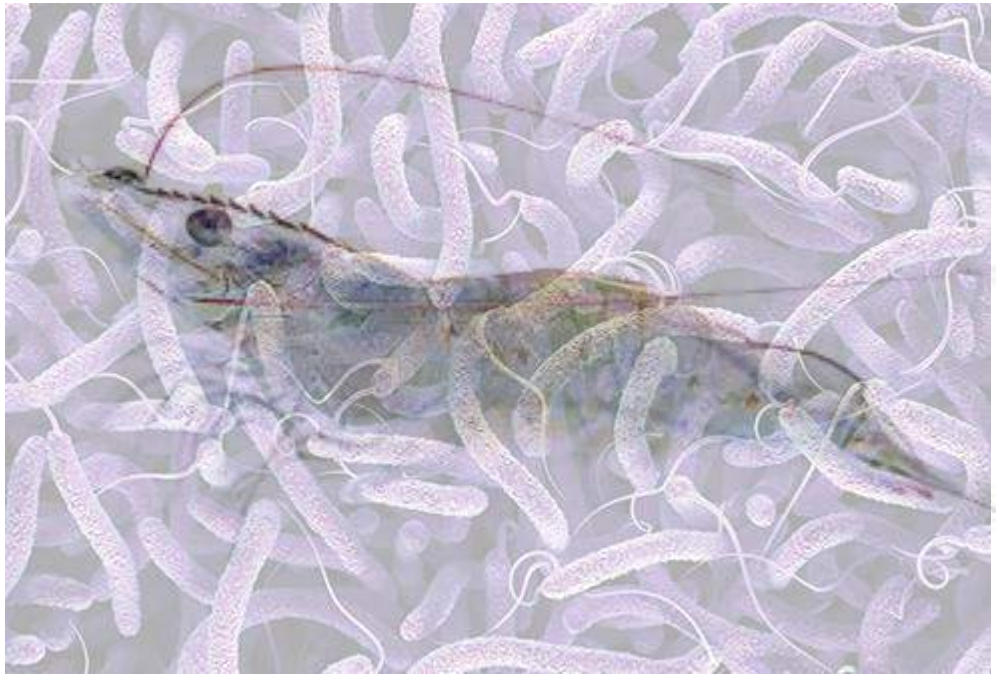
FHS Webinar 4: Small and Terrible! Significant Bacterial Diseases in Aquaculture

The gut as the first line of defense against bacterial diseases

Pikul Jiravanichpaisal

Fish and shrimp are constantly in contact with a complex and dynamic microbiota in their environment

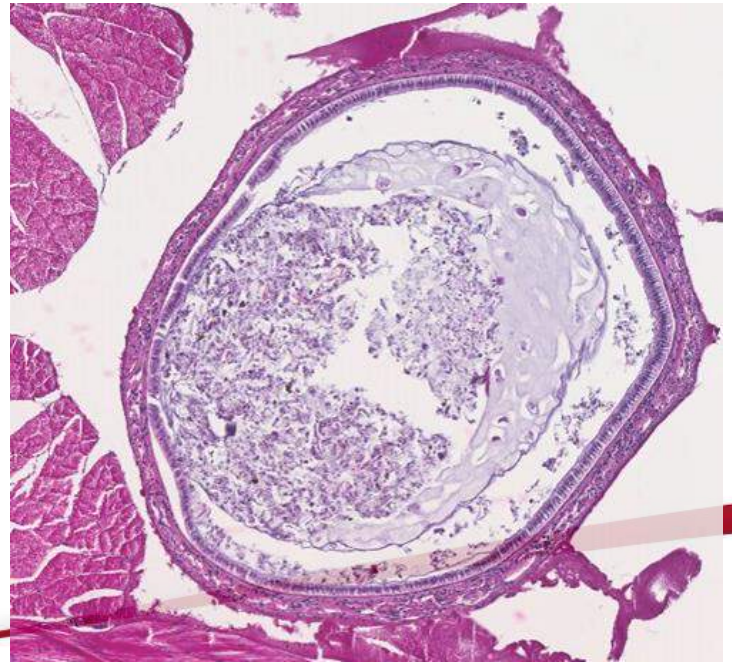


EMS/AHPND

Streptococcosis

The gut of animals is the largest and most important barrier against the external environment, which is made up of a single layer of epithelial cells.

The intestine is a multifunctional organ that is important for nutrient uptake, pathogen recognition and microbiome regulation.



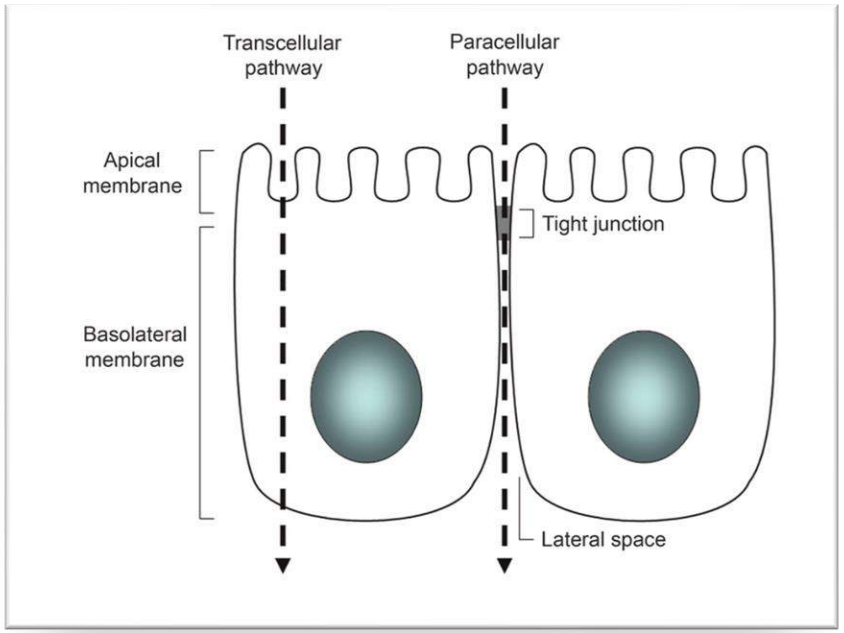
Potential pathogens mostly enter the host by one or more of three different tissue:

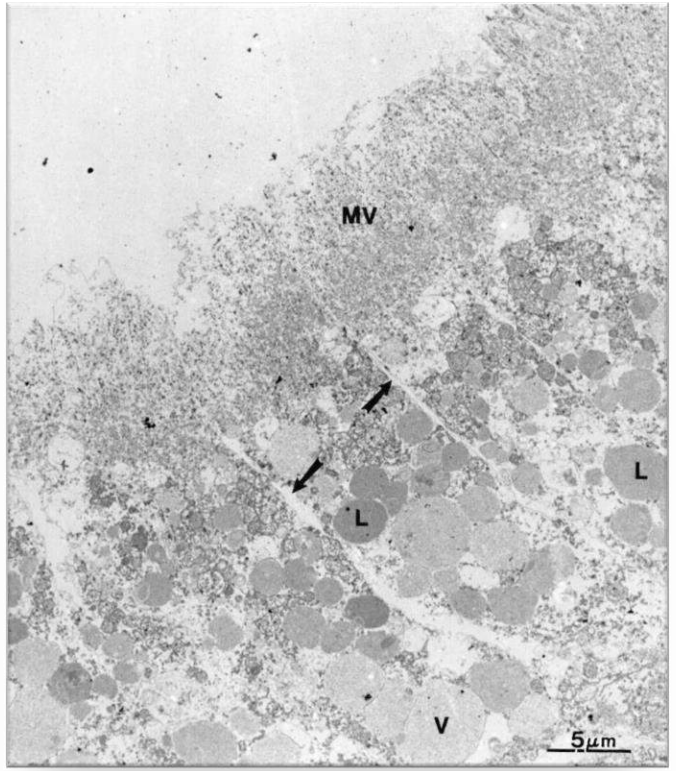
(a) skin/ shell

(b) gills

(c) gastrointestinal tract (GI tract)

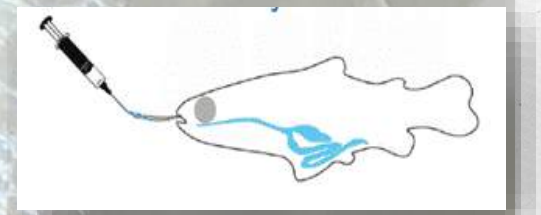
GI tract is an important entry point for pathogens





Bacteria can cross the epithelial cell in three different ways.

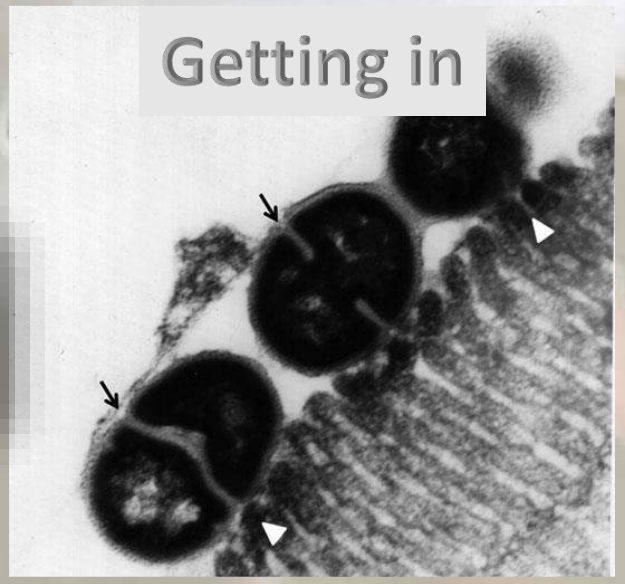
- Undamaged tissue, bacteria can translocate by transcellular or paracellular routes.
- Bacteria can damage the intestinal lining with extracellular enzymes or toxins before entering.

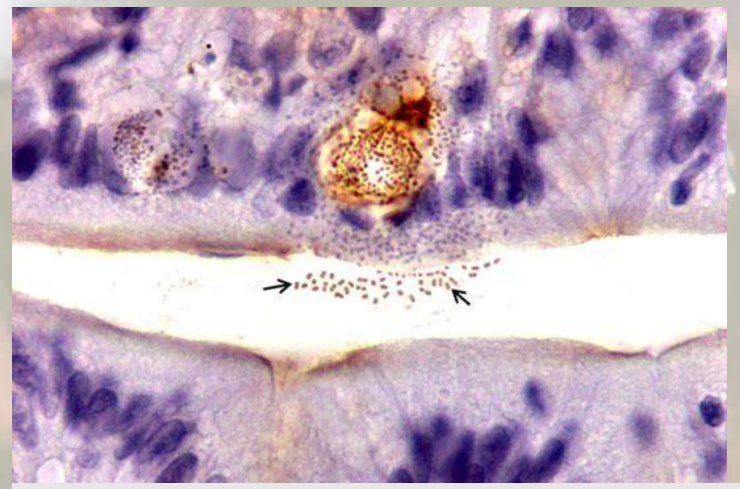


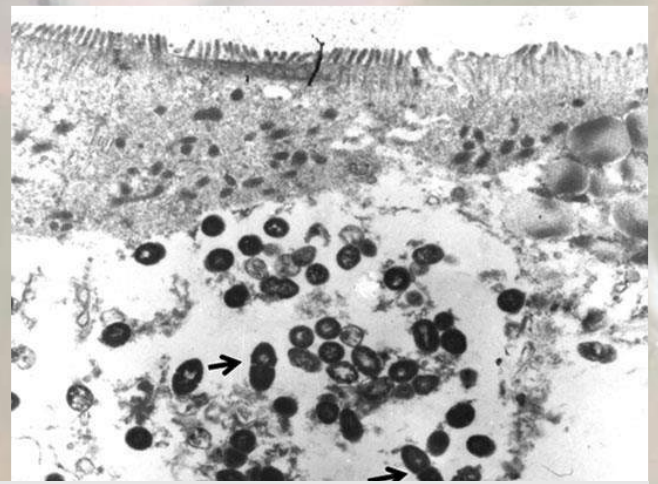
Gastric gavage

Streptococcosis

Getting in







Staying in & Defeat the host defense

Iregu, C.A., et al., Journal of Fish Diseases 2015

Damage the tissue

Exit

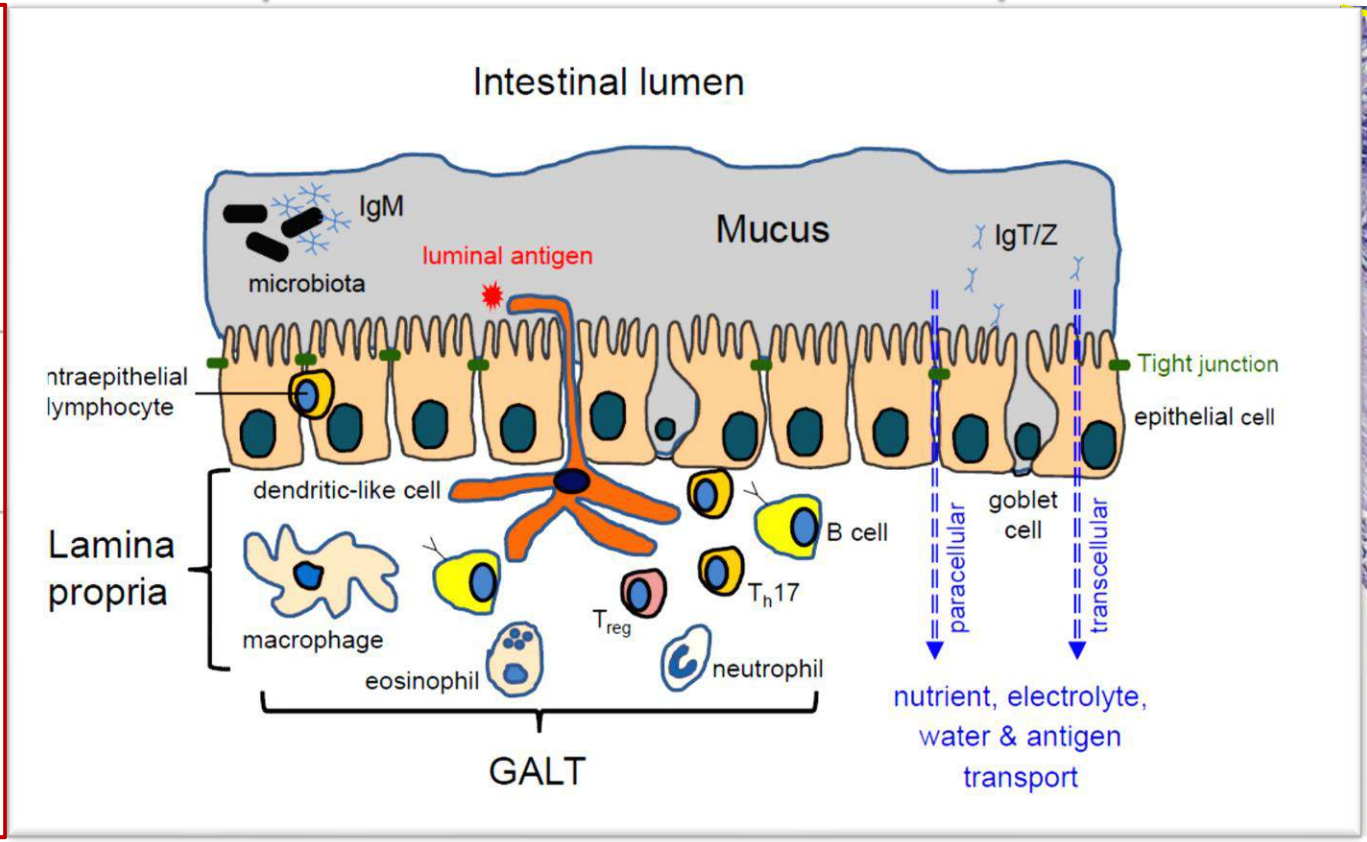
When fish are exposed to pathogen, what do they need ?

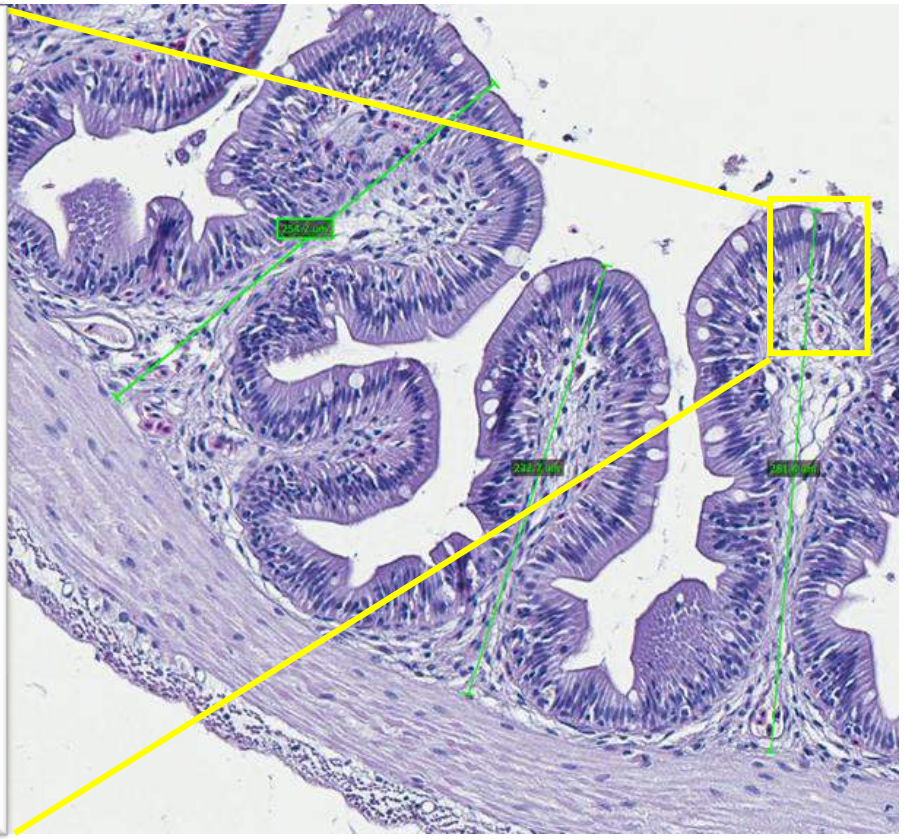
A protective barrier to keep unwanted out.

Biochemical barrier

Physical barrier

Immunological barrier



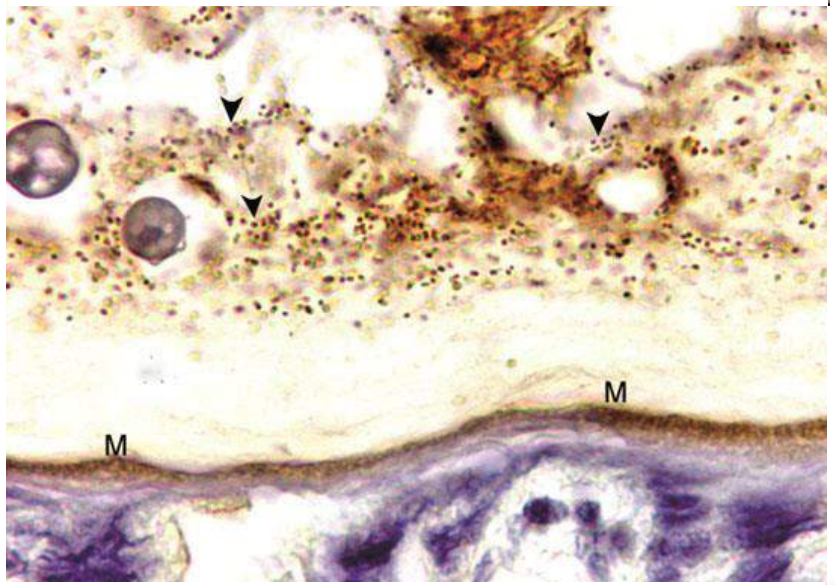


Immunological barrier	Physical barrier	Biochemical barrier

Mucus

The host employs mucus in several ways to protect the inner epithelia of the body from invading microbes.

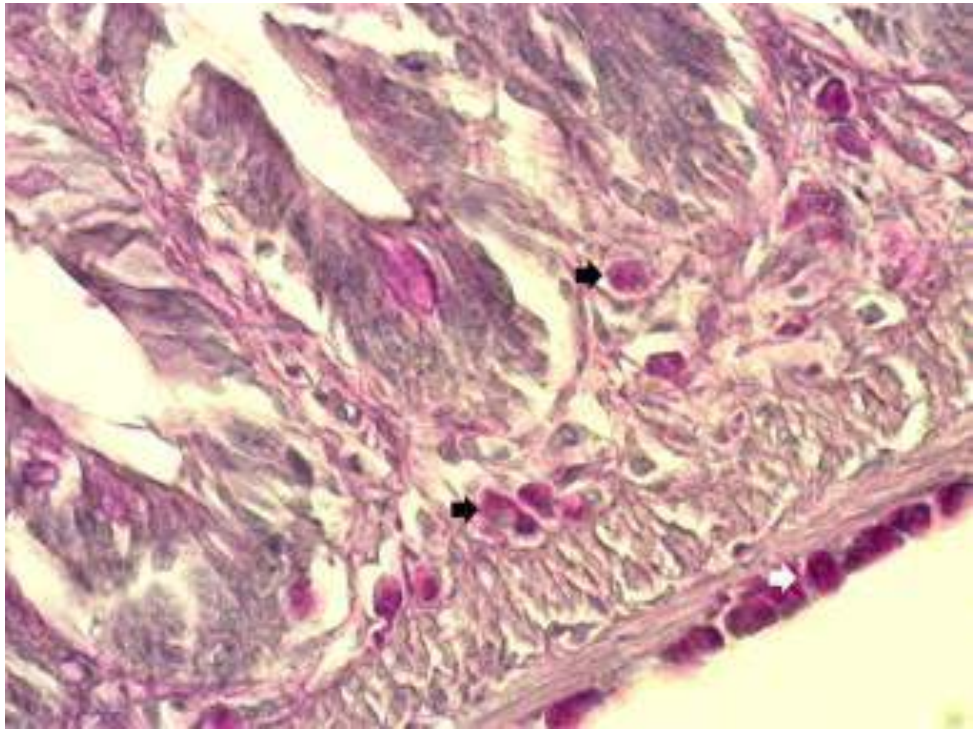
A biological mesh of mucus



Severe mucus secretion in the gastric and intestinal lumen of tilapia exposed to *S. agalactiae*

Iregui C.A., et al., 2015. Fish Disease

The antimicrobial peptides & Enzymes

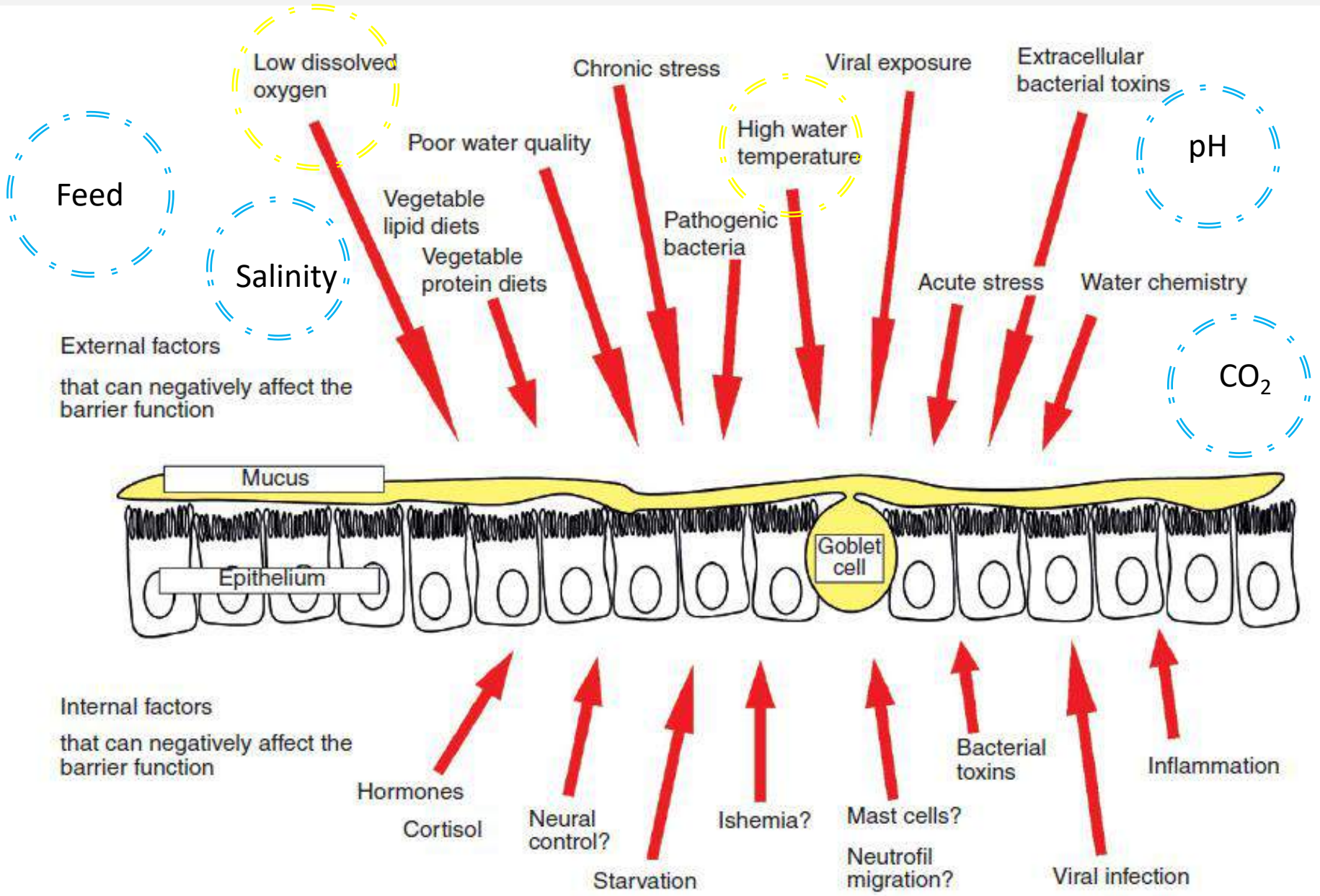


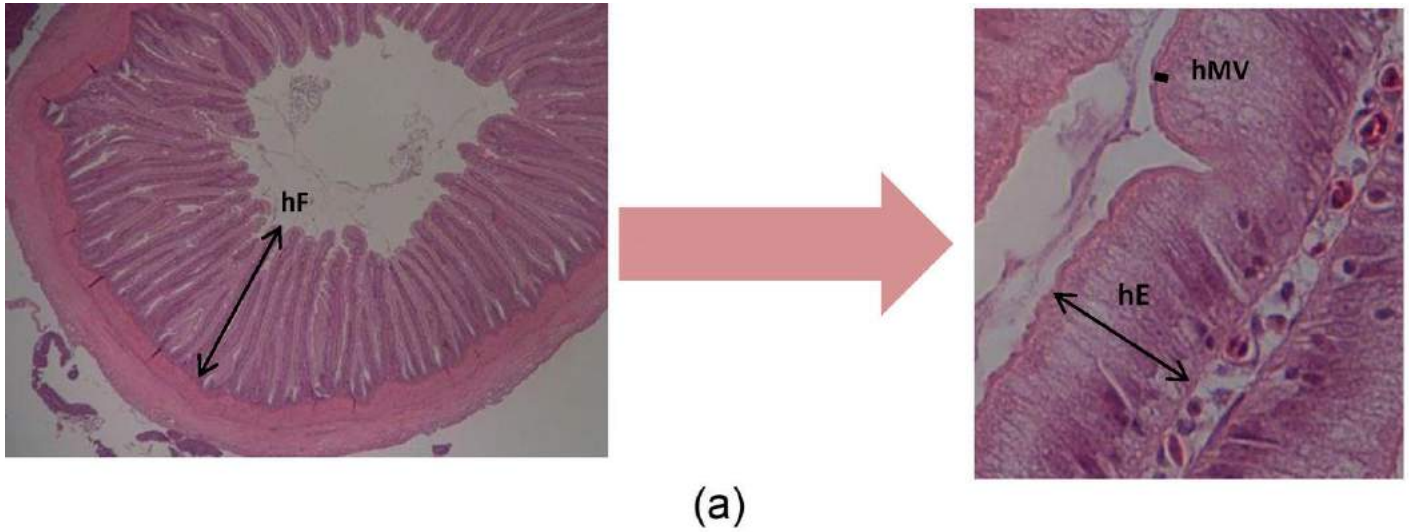
MC localization in the submucosa of the digestive tract (black arrow).

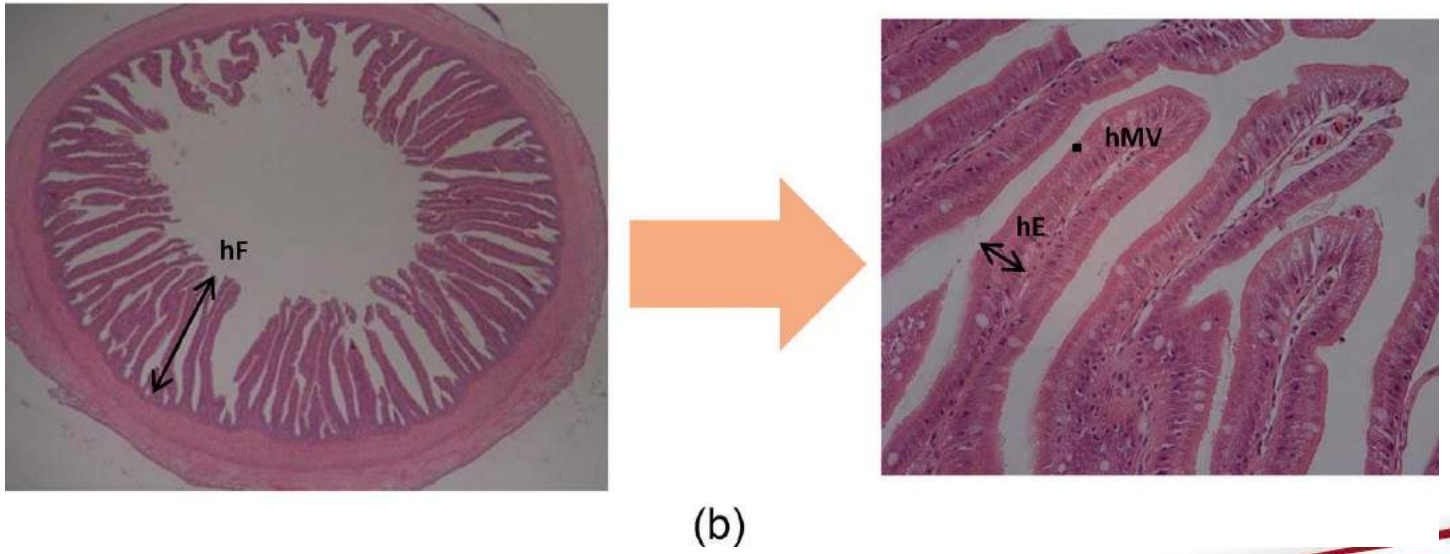
EGC/MCs (Eosinophilic Granules Cells/Mast cells) contain alkaline phosphatases, arylsulphatase and 5-nucleotidase, lysozyme, peroxidase,
And antimicrobial peptides such as piscidins

Why do fish get sick while having a well-developed immune system?

Factors that disrupt the integrity of the intestinal epithelium and reduce the barrier.







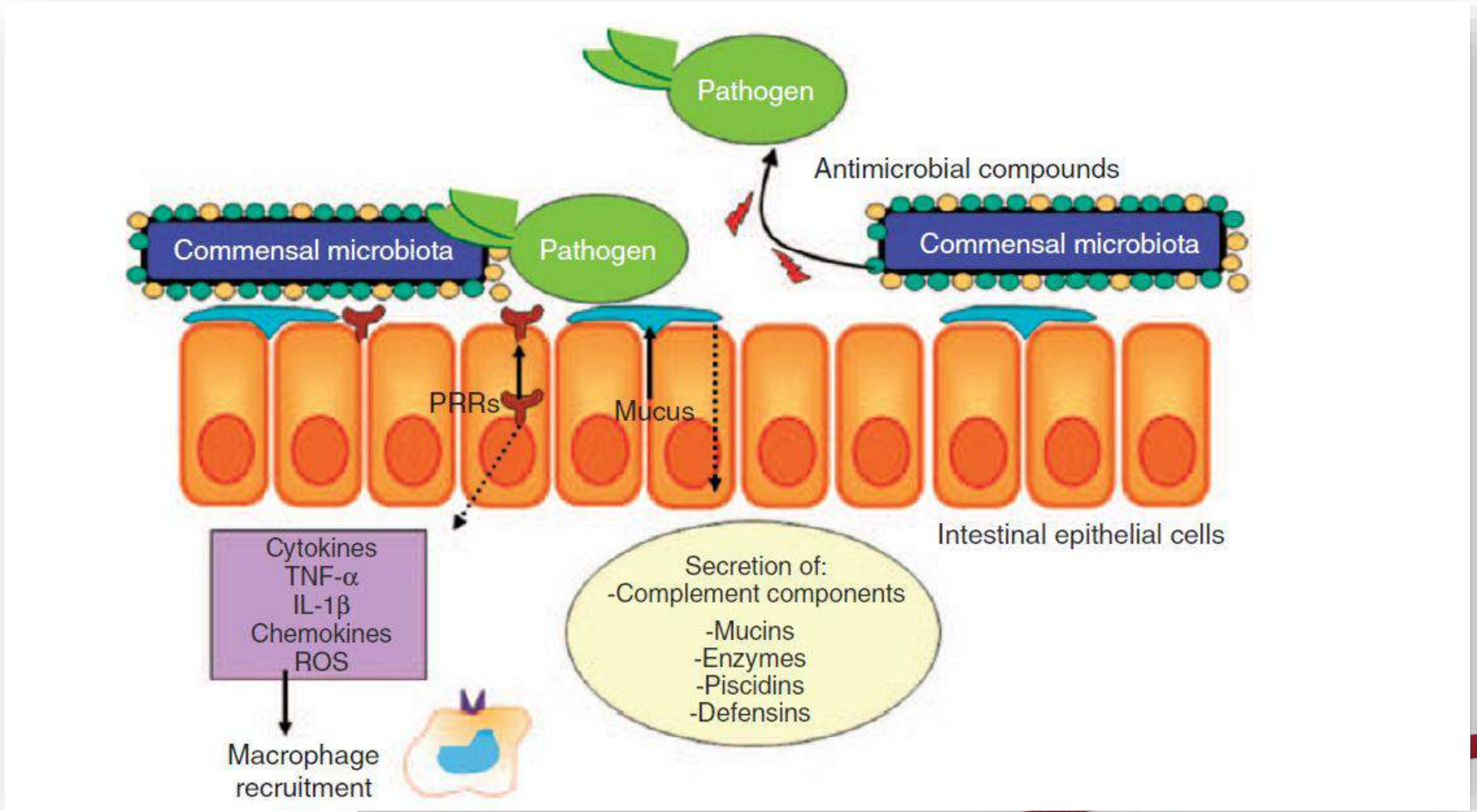
Dietary soybean meal can cause a dose-dependent type of distal intestine inflammation called enteritis in commercial fish species including salmonids. This leads to reduced absorptive capacity, increased mucus secretion, hyperpermeability.

Kumar V., et al. 2020. Soybean for Human Consumption and Animal Feed-IntechOpen

Commensal microbiota serve as an important first-line defense against invading pathogens.

Commensal bacteria can give protection by producing inhibitory chemicals, competing for adhesion sites, or regulating the immune response to make a hostile environment for pathogenic bacteria.

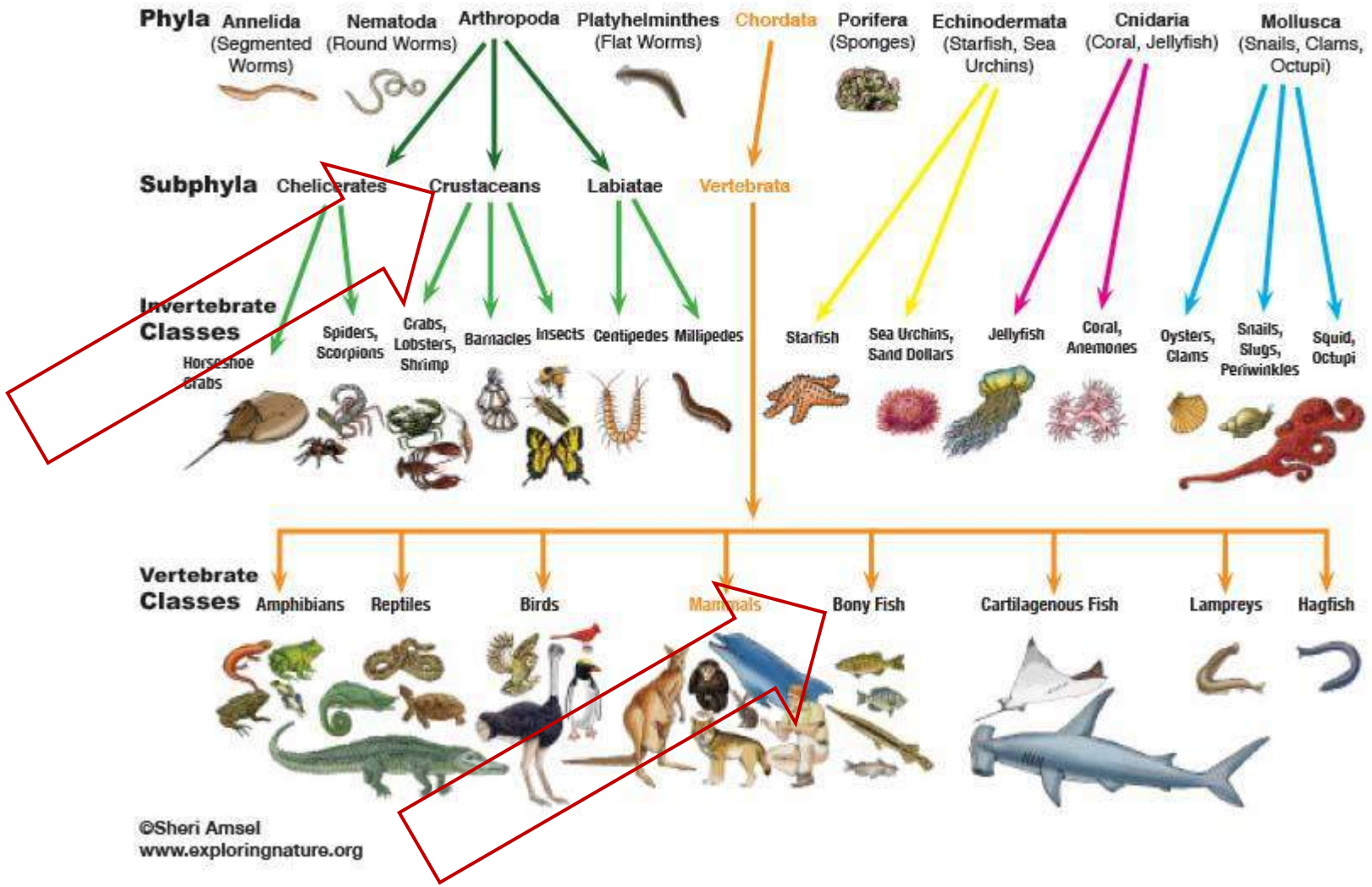
Host-microbiota interactions in fish gut

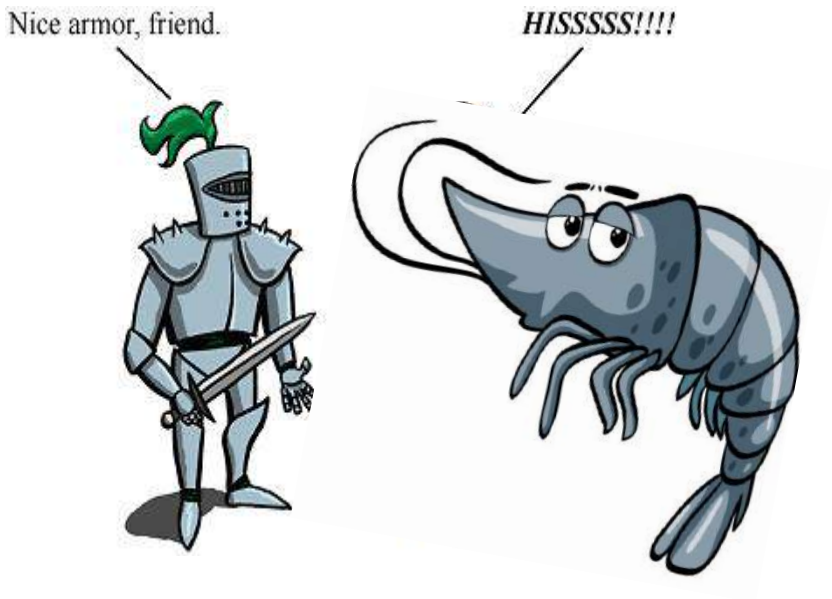


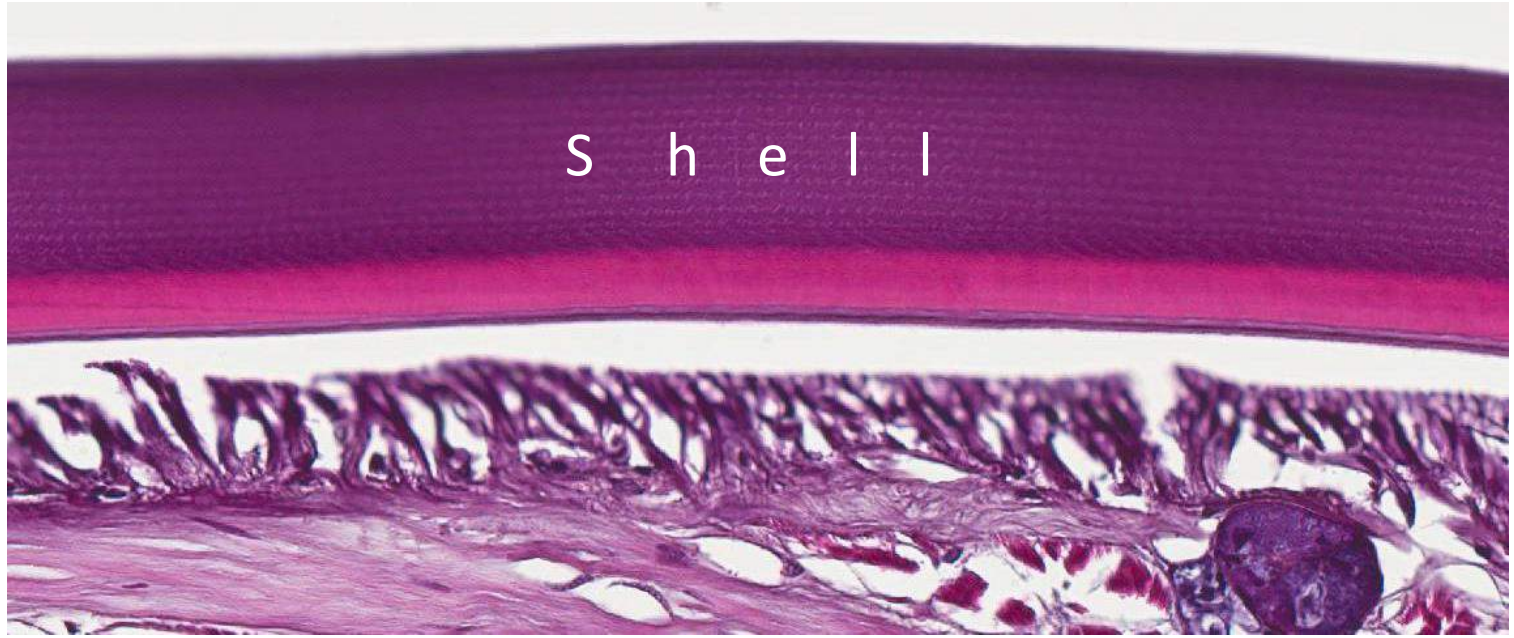
Host–microbiota interactions within the fish intestinal ecosystem

T Pérez[1], JL Balcázar[2], I Ruiz-Zarzuela[1], N Halaihel[1], D Vendrell[1], I de Blas[1] and JL Múzquiz[1]

Animal Kingdom

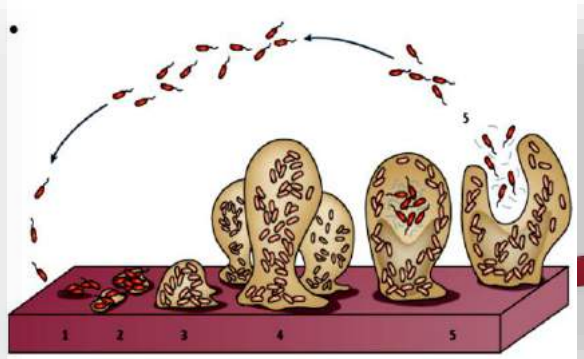






The shrimp gut’s first line of defense: simple but efficient

The habitat of Vp in the shrimp farming system

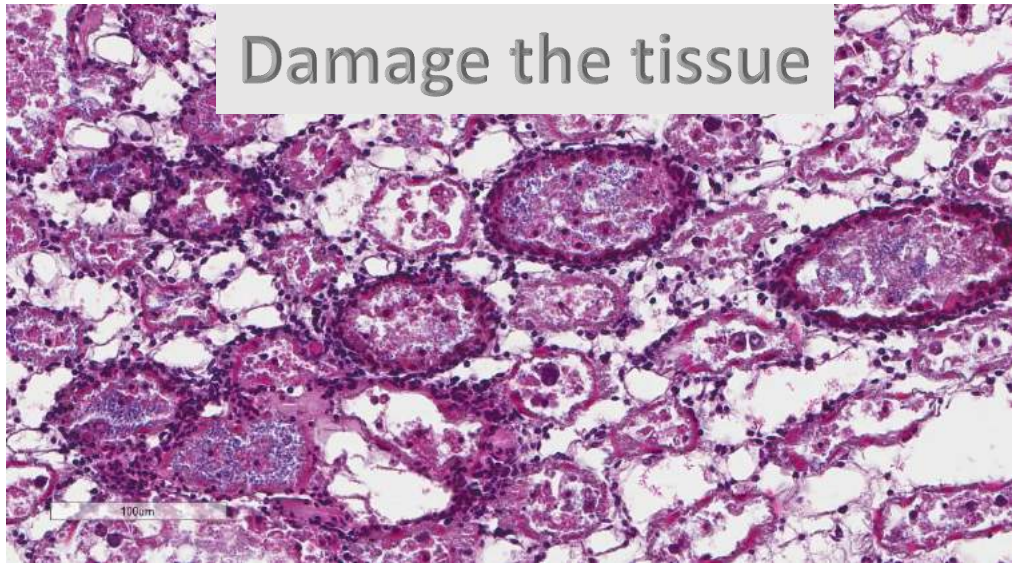


Biofilm

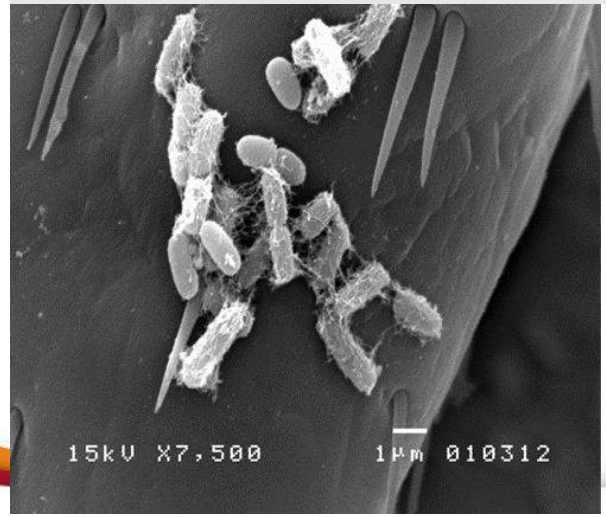
Bacterial/AHHPND infection process

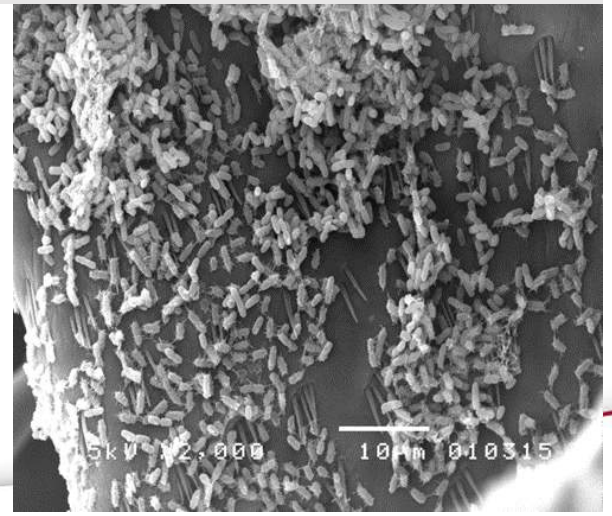
Getting in

Damage the tissue

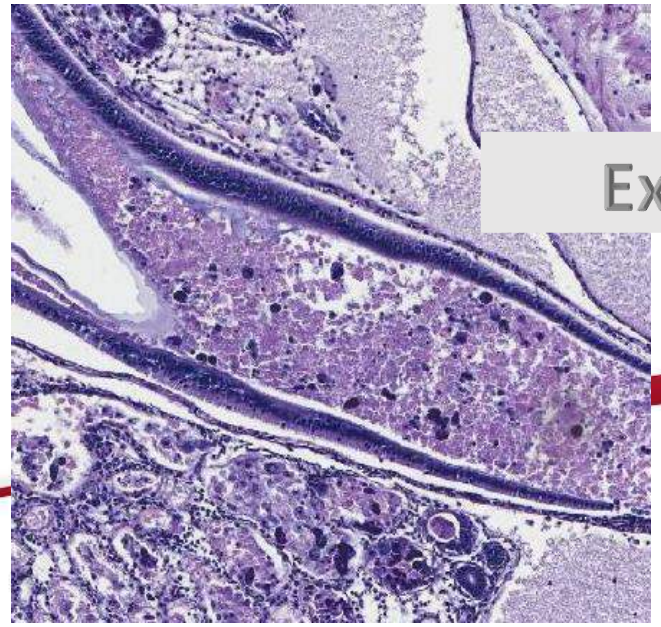


Staying in & Defeat the host defense

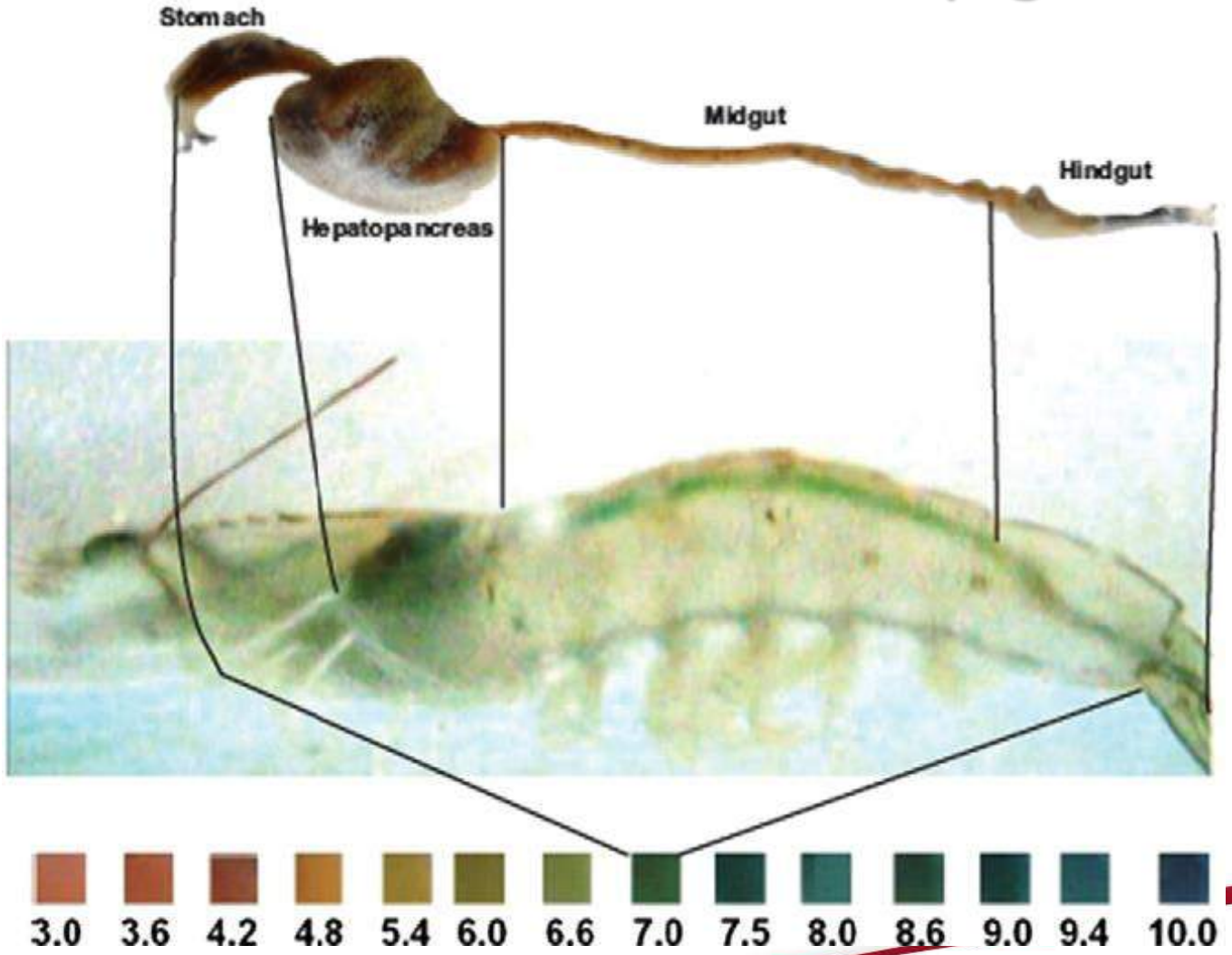




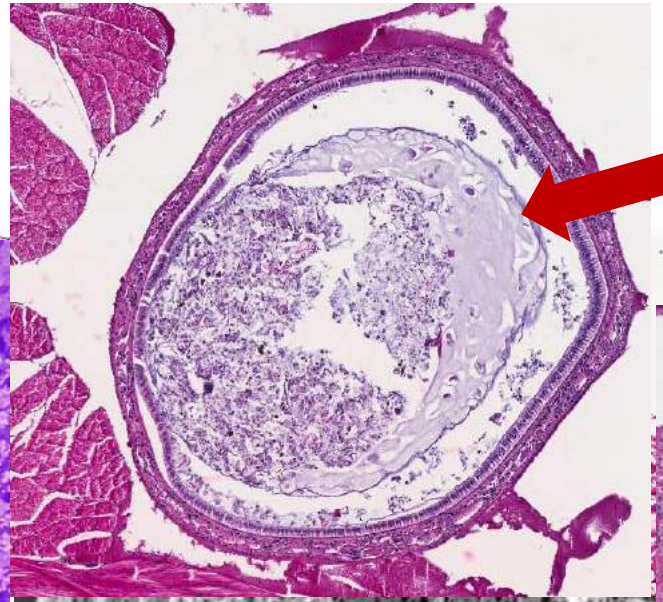
Exit



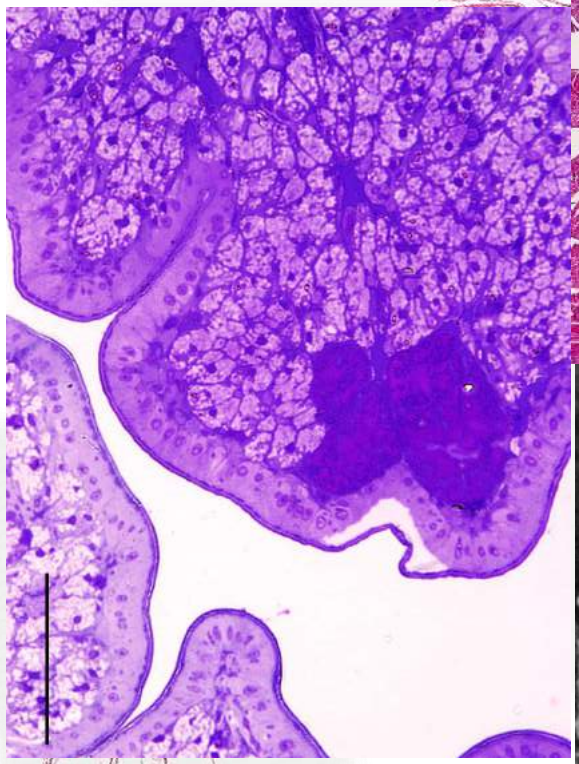
Shrimp gut



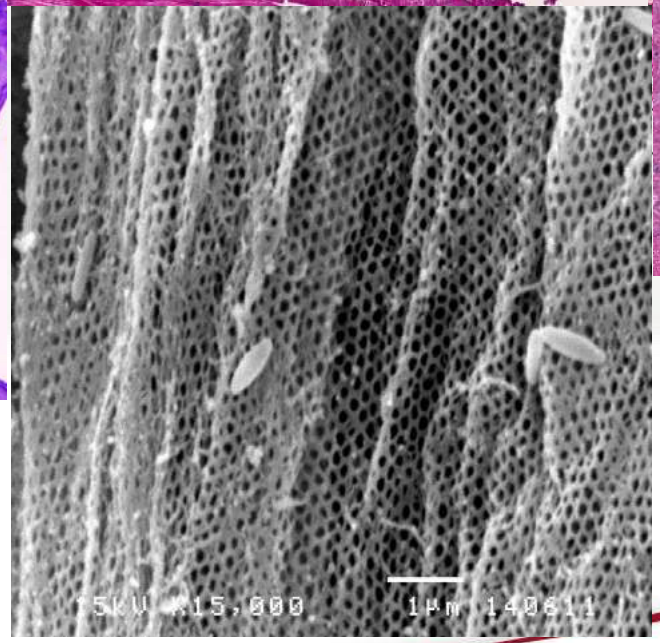
D. Alexandre et al. / Comparative Biochemistry and Physiology, Part B 172–173 (2014) 90–95



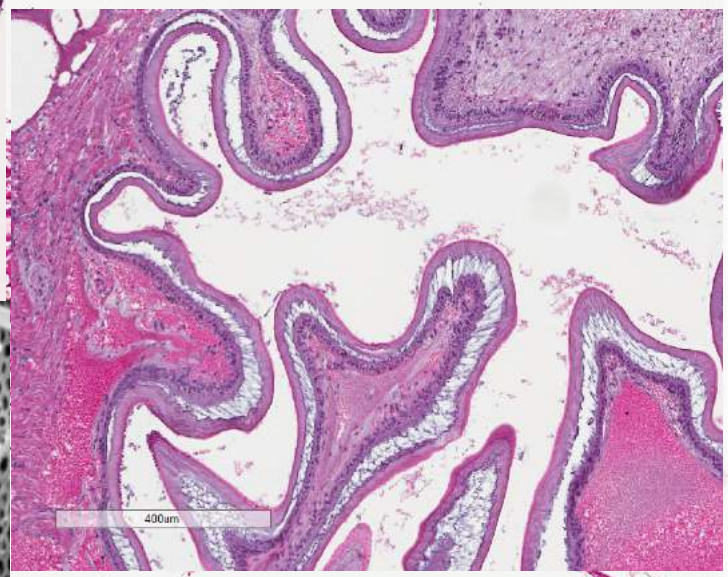
Peritrophic matrix



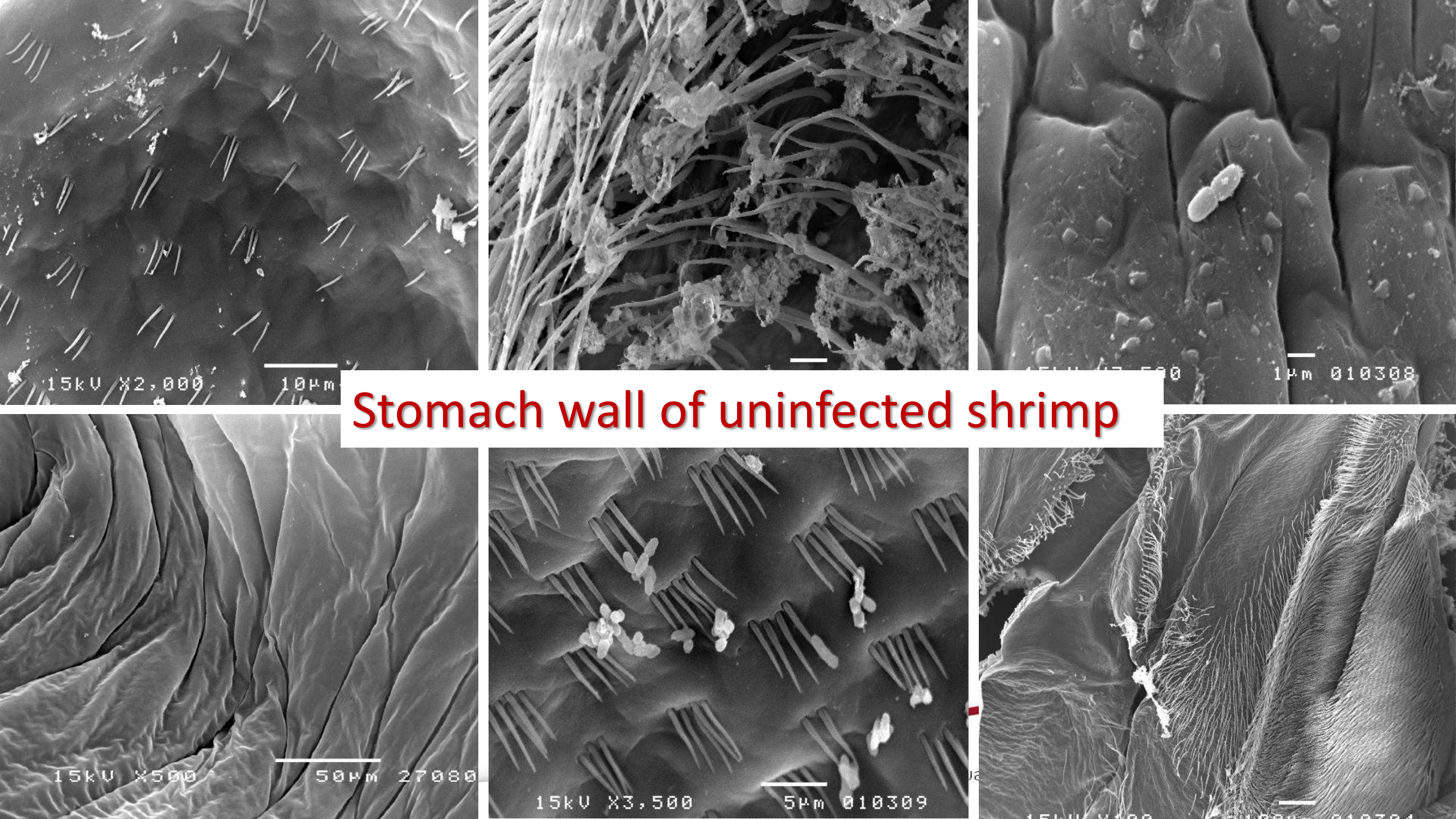
Hindgut



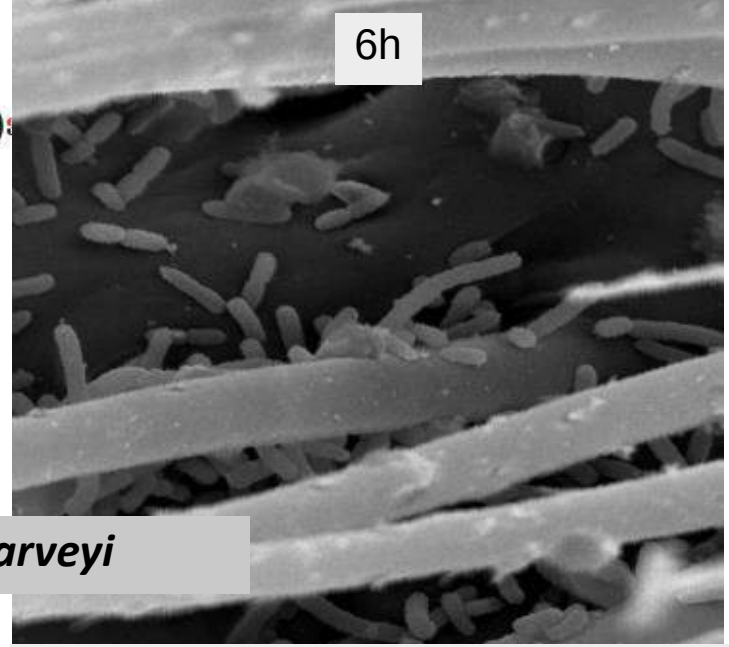
Midgut

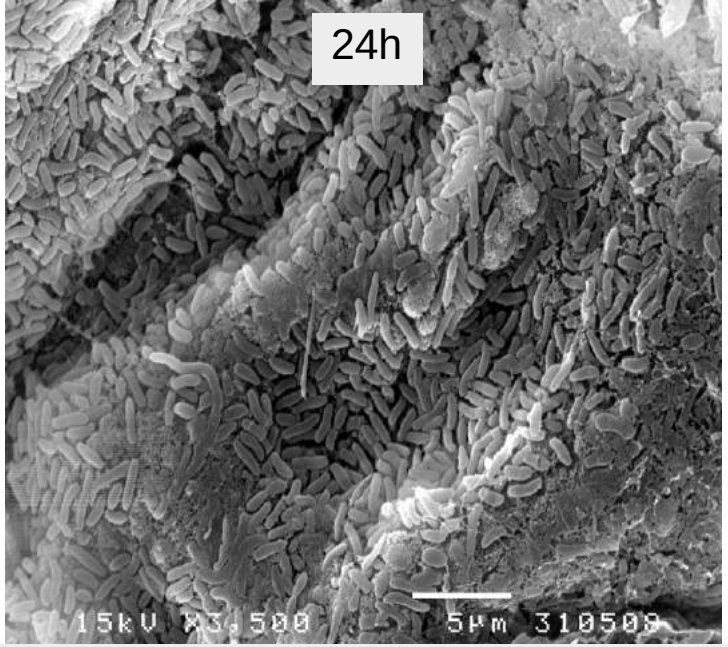


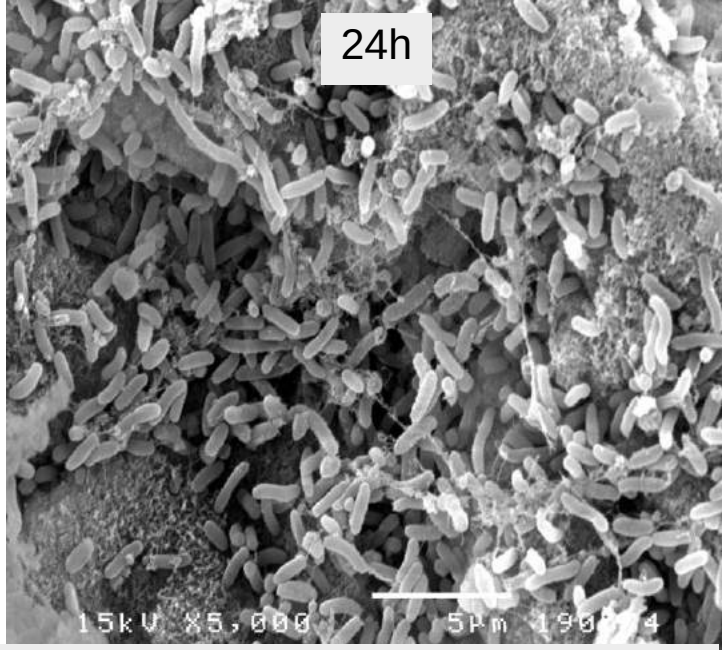
Foregut



Stomach wall of uninfected shrimp





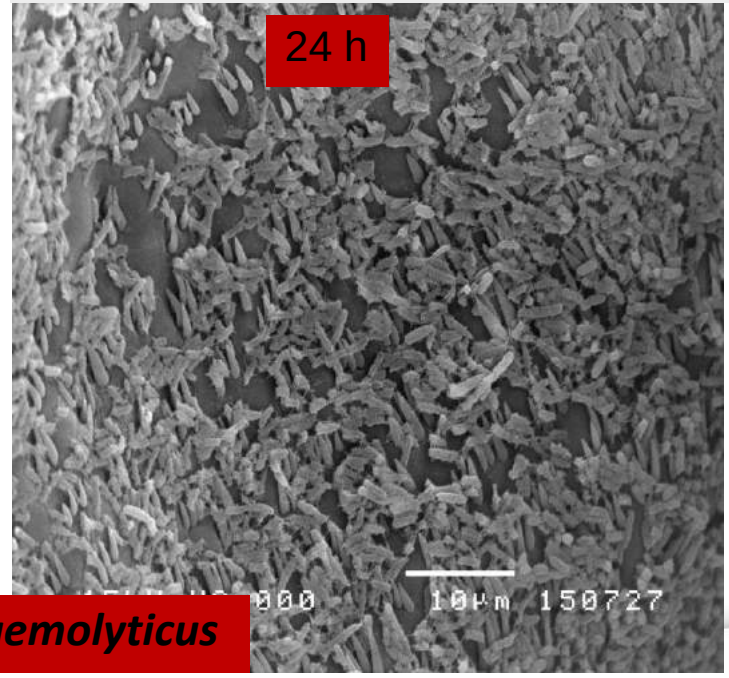


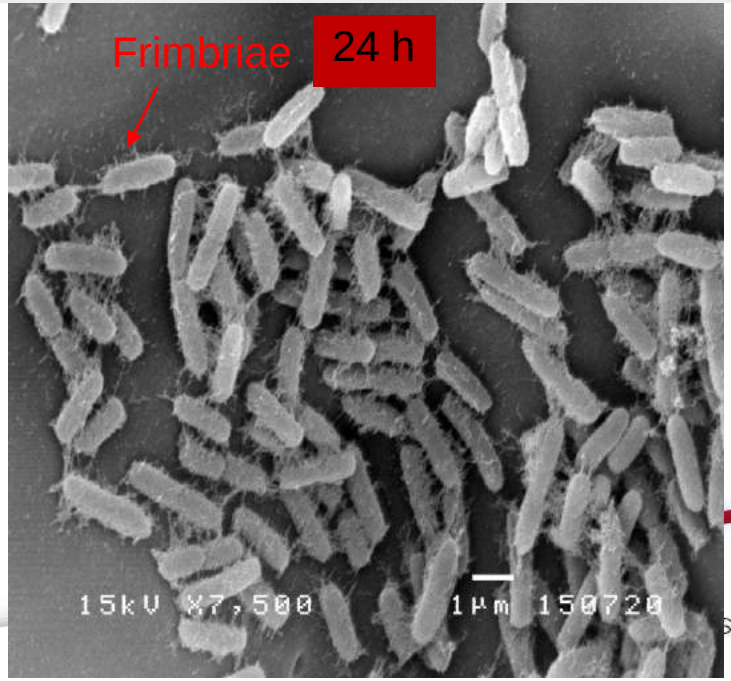
V. harveyi

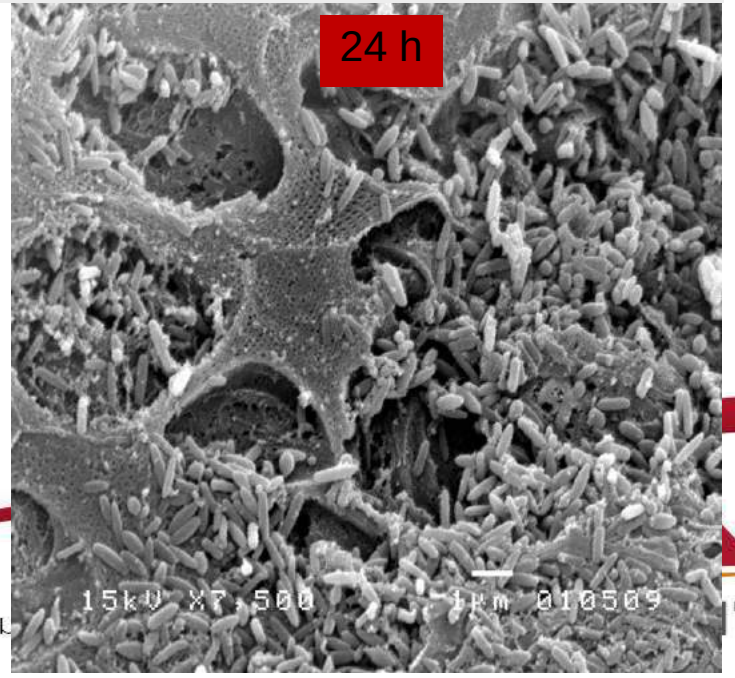
Stomach

Stomach

Midgut

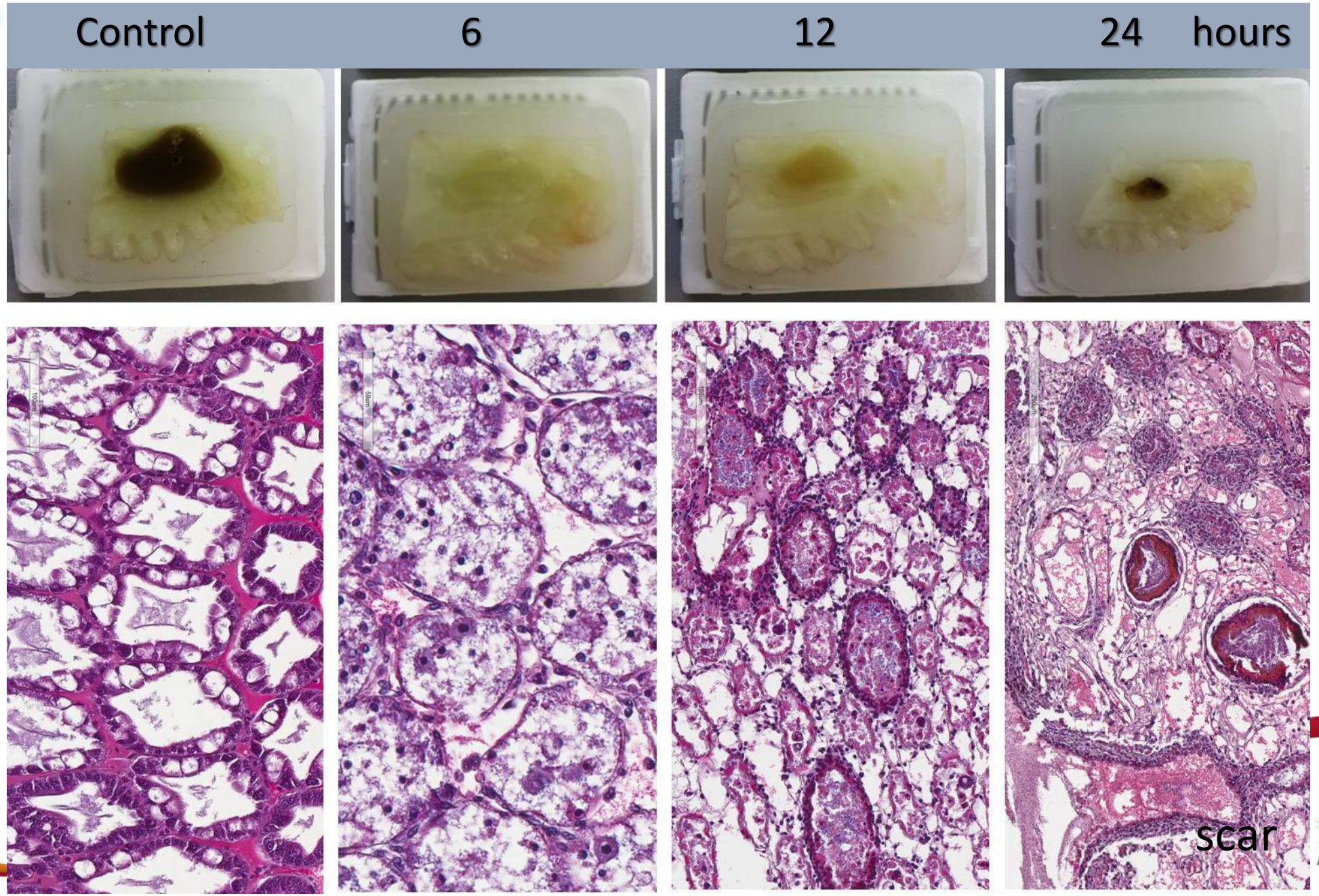




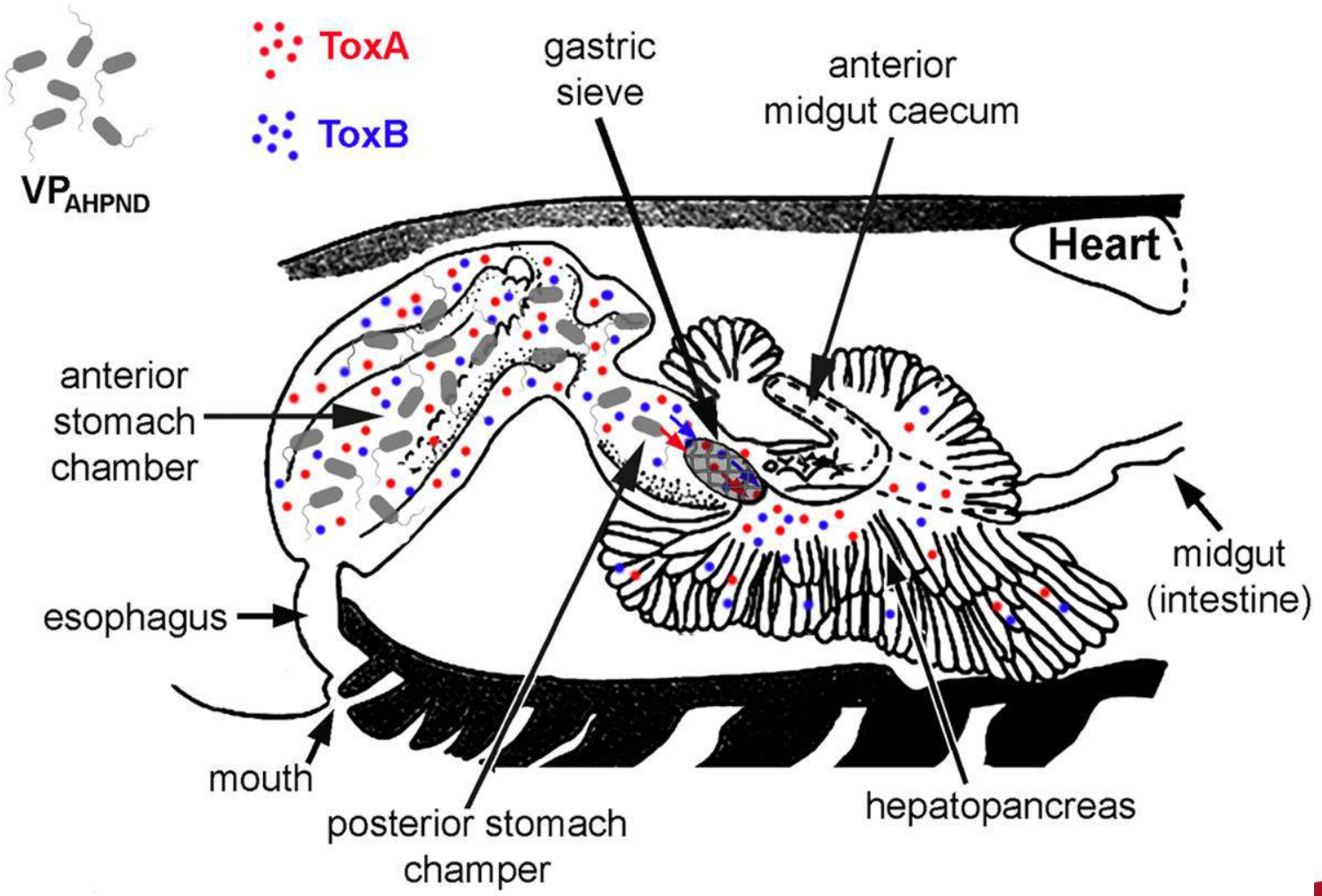


V. parahaemolyticus

AHPND-*Vp* damage the hepatopancreas



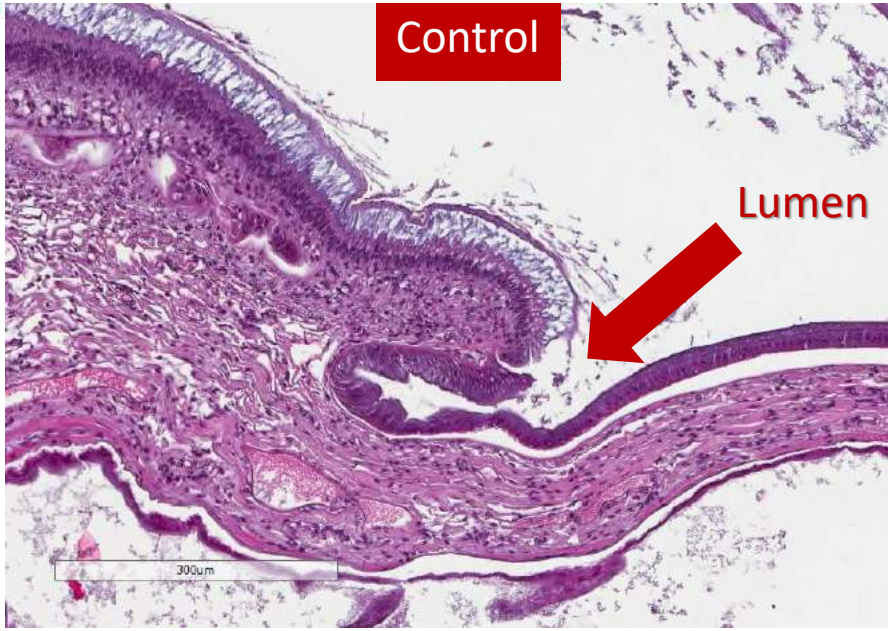
Update on early mortality syndrome/acute hepatopancreatic necrosis disease by April 2018

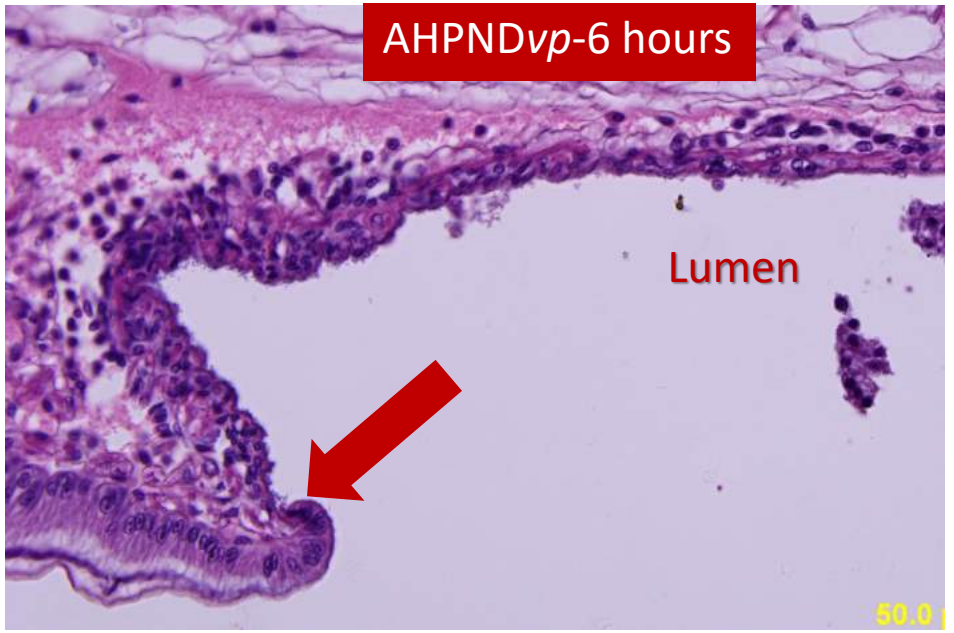


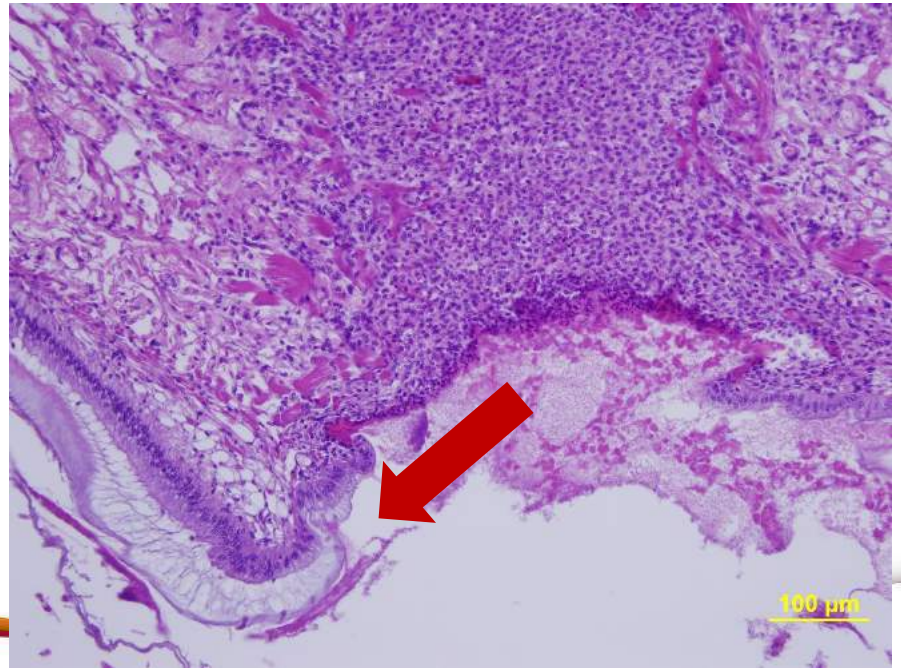
Prachumwat A., et al. J World Aquacult Soc. 2019;50:5–17.

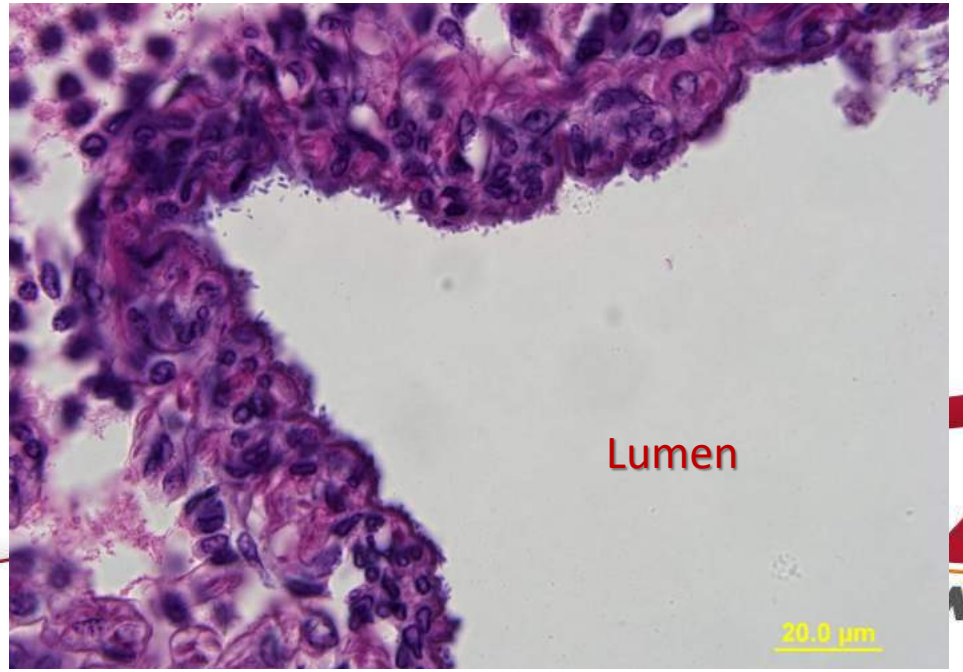

800um

The junction between stomach and midgut







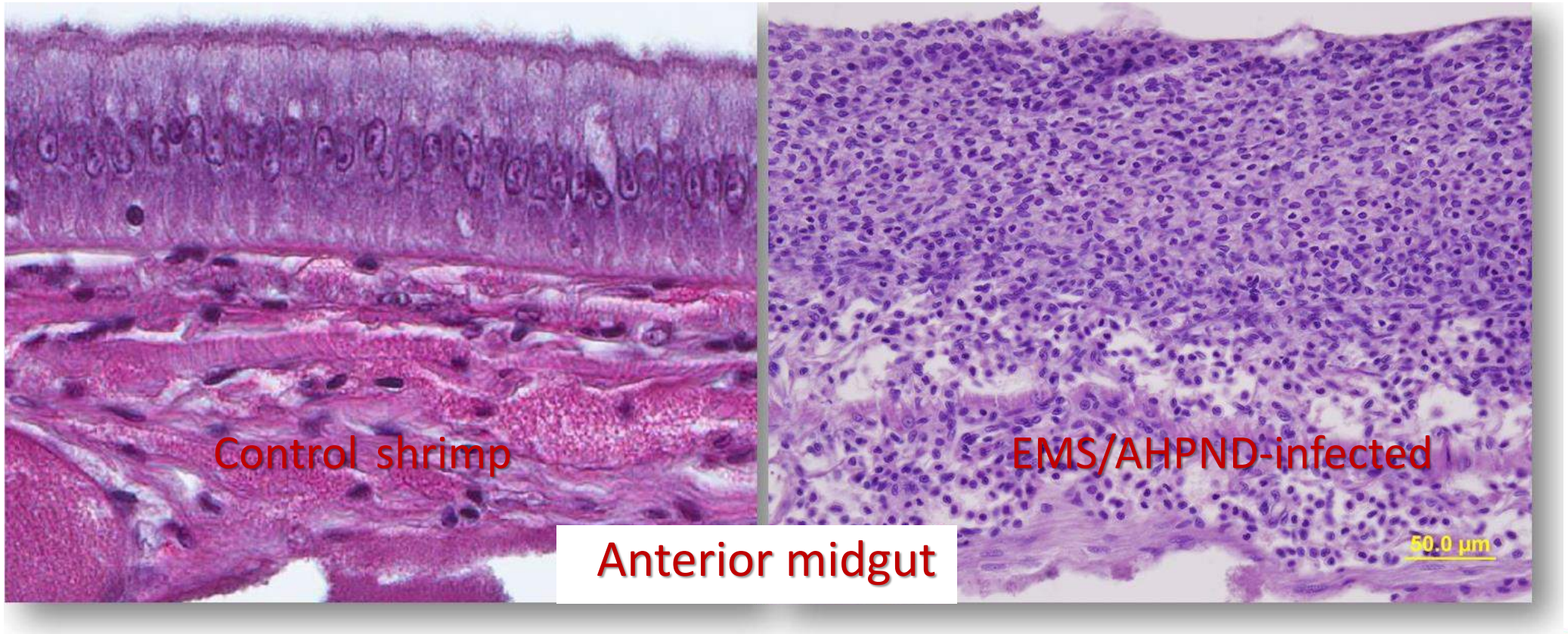


The first line of defense

Optimal goal: Prevent microorganisms from gaining access

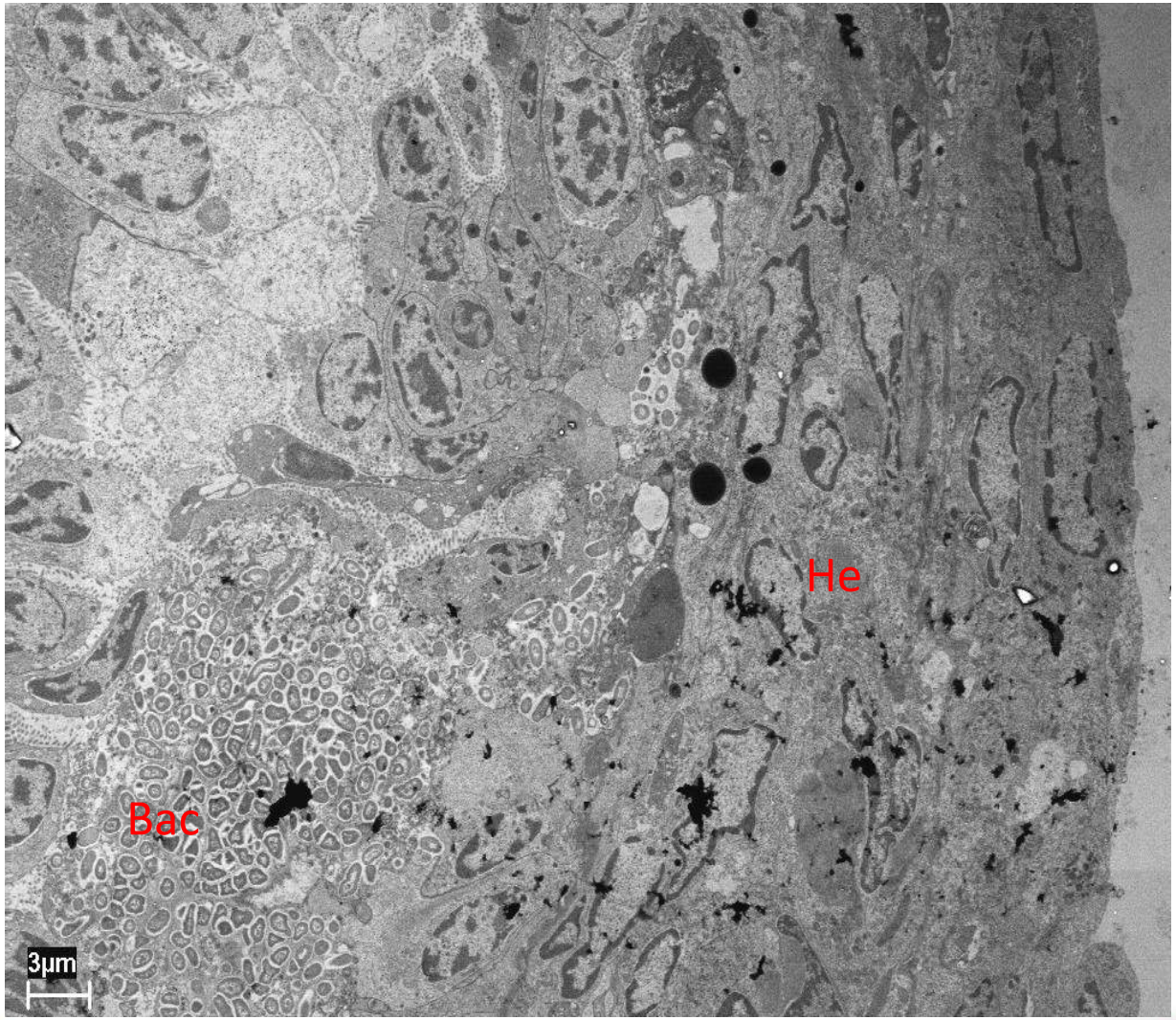
- The exoskeleton or cuticle (stomach and hindgut)- Form an efficient barrier protecting against invasion by potential pathogens
- Molting
- Peritrophic matrix
- Cellular response (Phagocytosis, encapsulation etc.)
- Antimicrobial peptides
- Melanization
- Intact normal flora

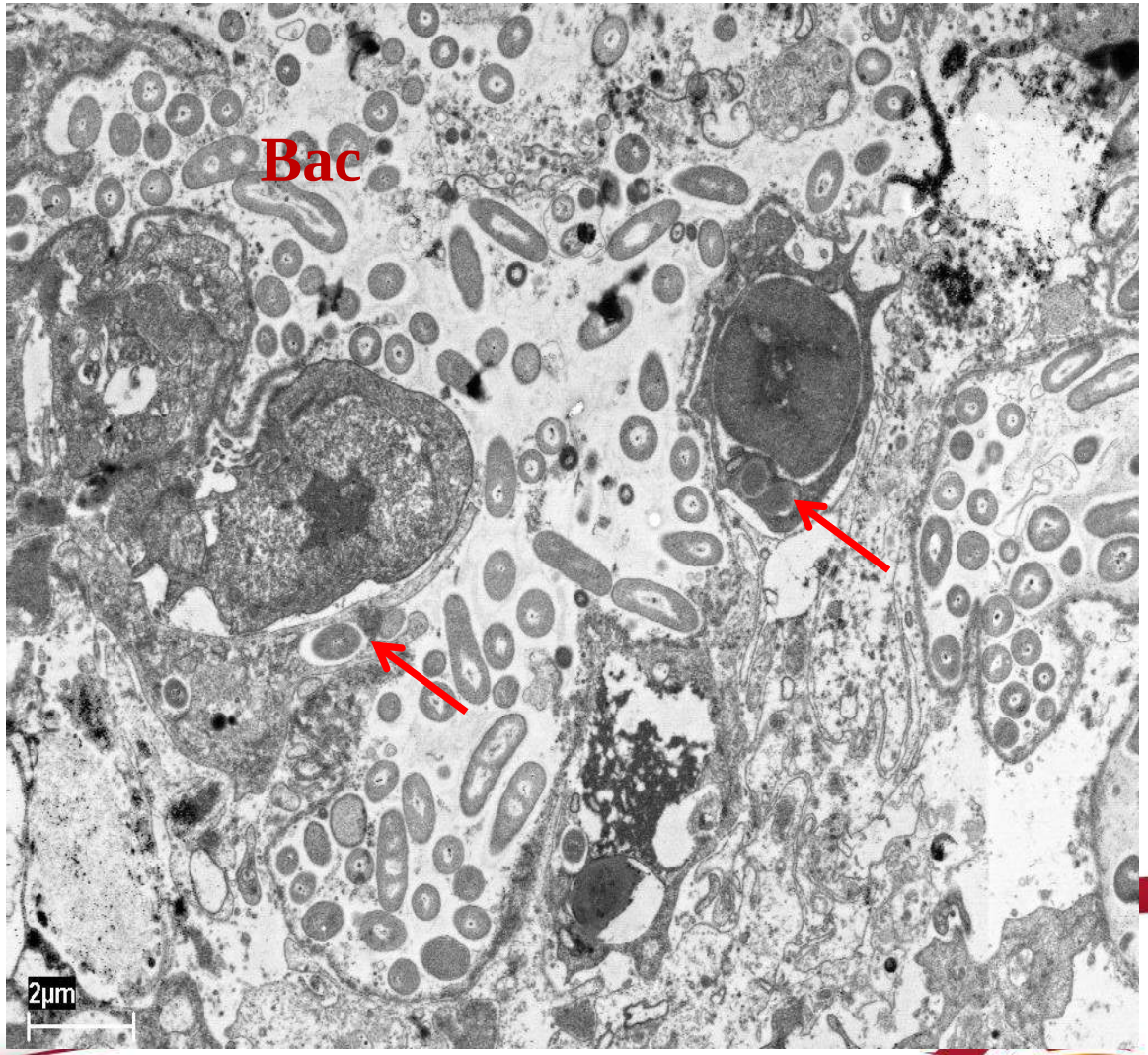
Cellular response



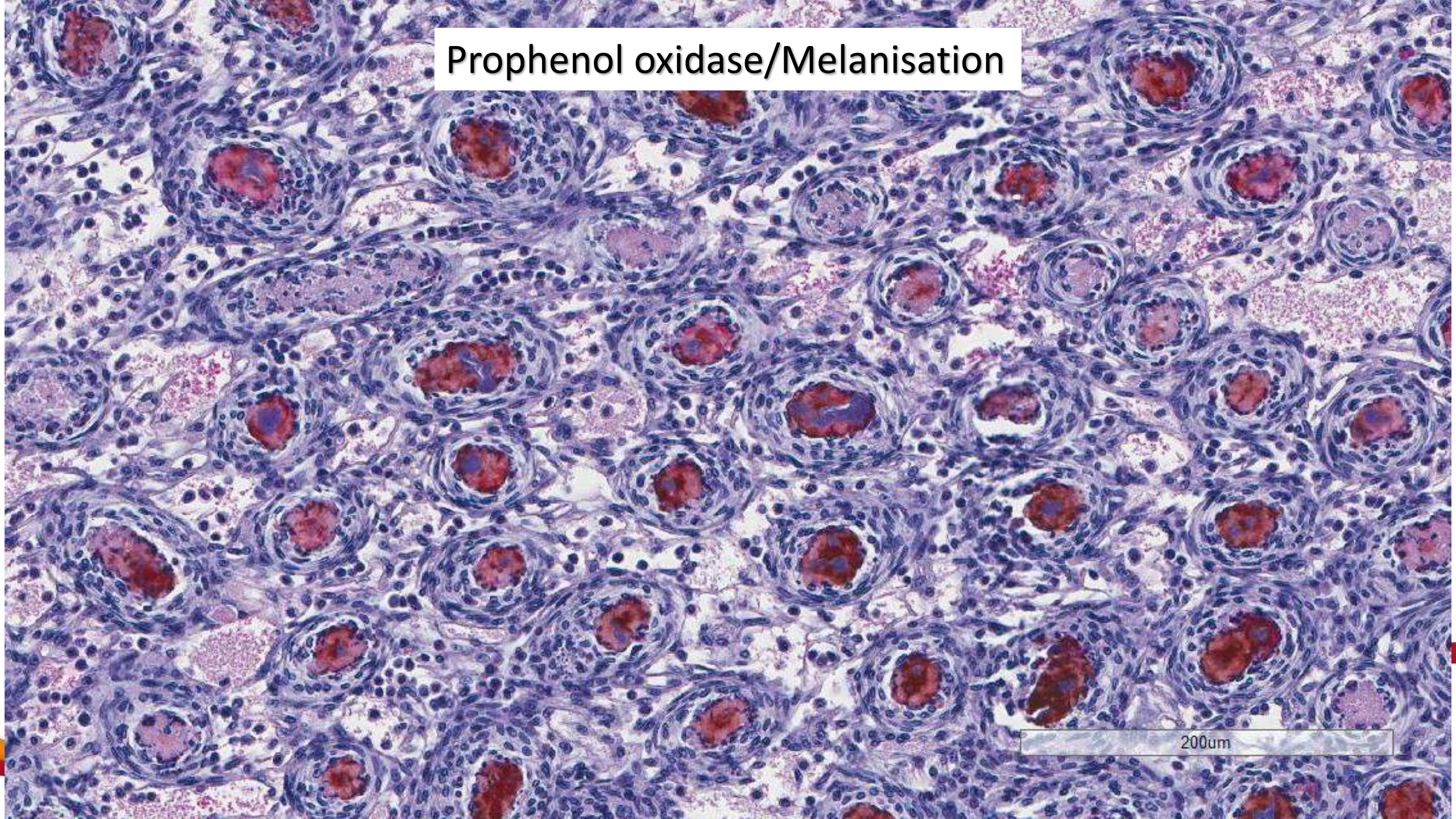
To prevent bacteria from entering the body, the cellular reaction is effective.

Cellular response (Haemocytes) and phagocytosis





Prophenol oxidase/Melanisation



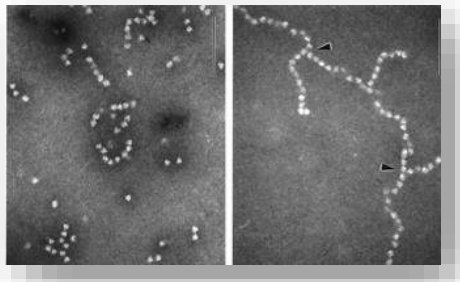
The last line of defense or haemocoelic internal defense

Optimal goal: Neutralized and destroy invaders

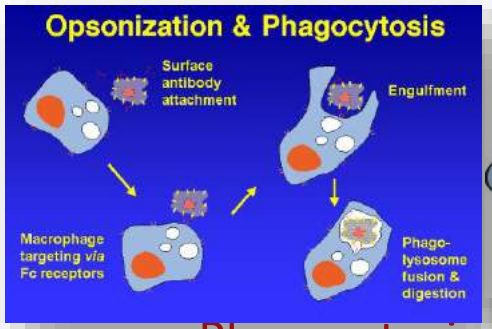
Complex network of cellular and humoral responses that work together to protect the body

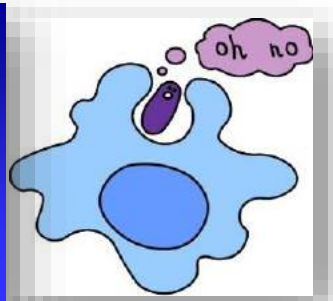
The last line of defense or haemocoelic internal defense

Gut lumen

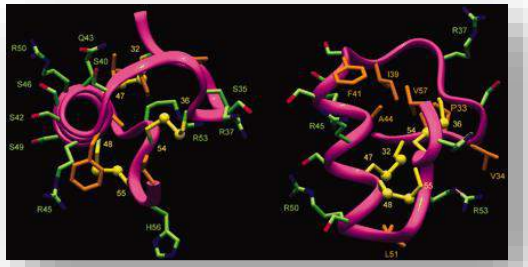


Clotting protein

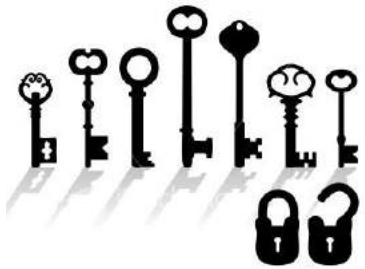




Phagocytosis

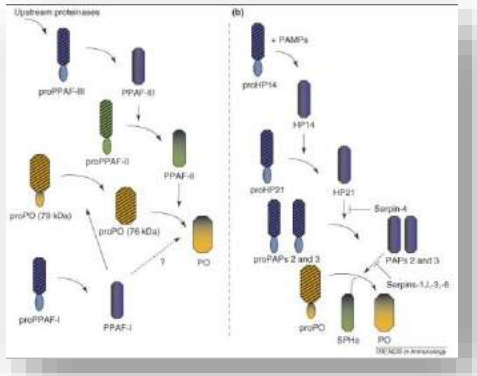


Antimicrobial peptides

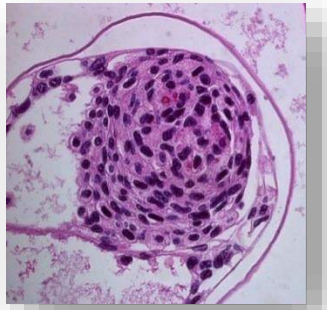


Pattern recognition protein

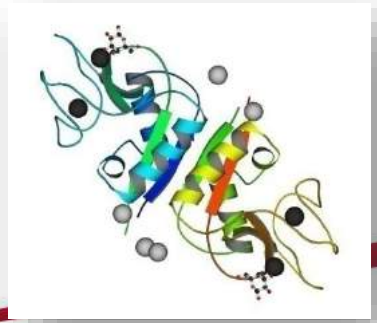
Antiviral defense



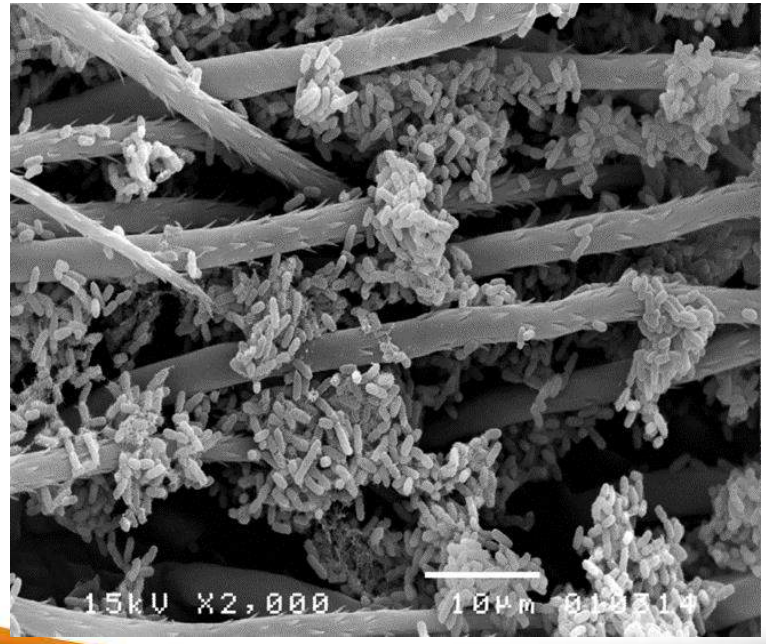
ProPO



Ecapsulation/ nodule formation



Lectins



Molting as a component of cuticle defense

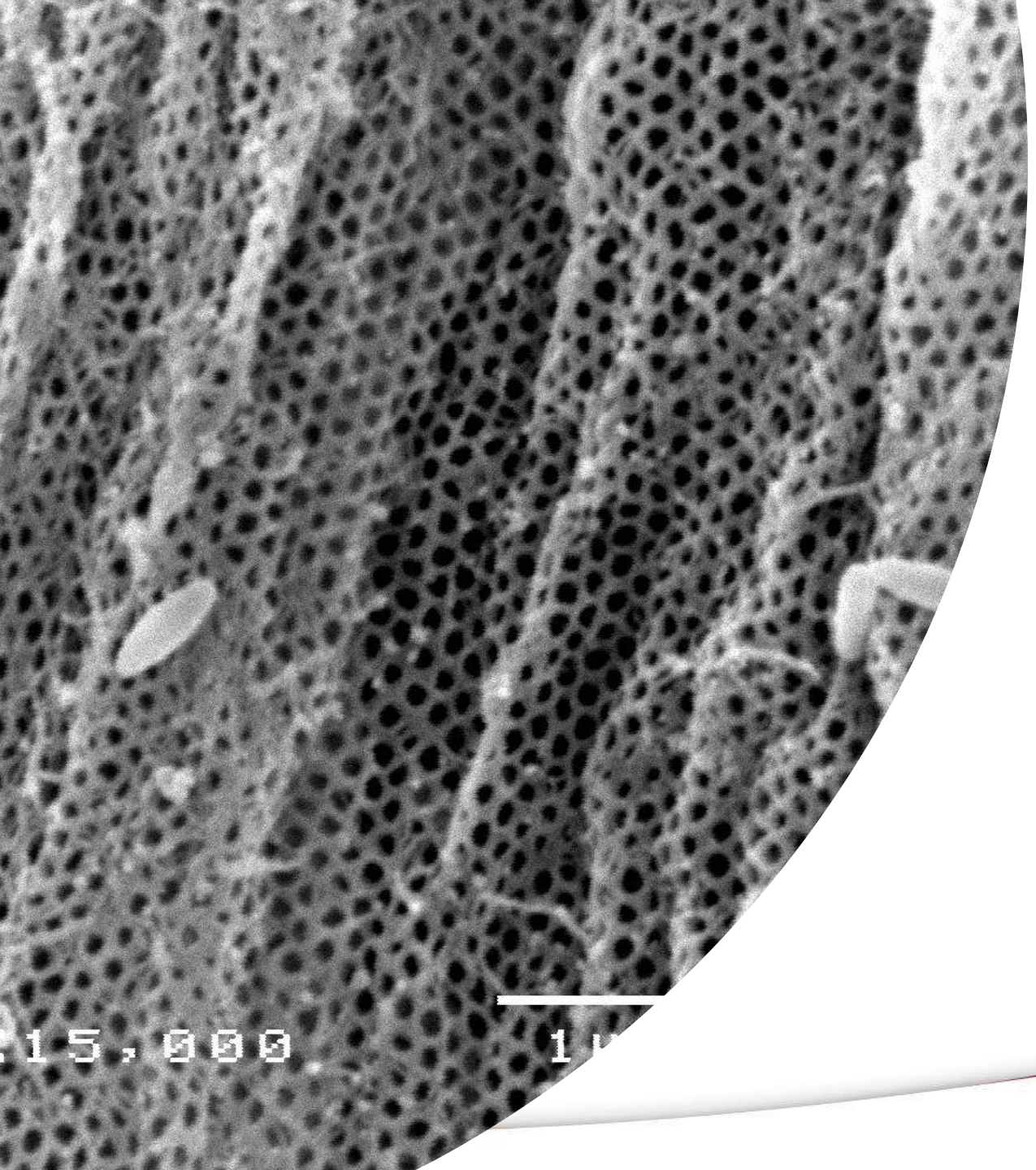
- Reducing the negative effects of wounding or bacteria from the cuticle
- Helps to remove bacteria/parasite attached to the cuticle and reduces the possibility of successful infection

Table 1 Average durations of the major moult stages and total moult cycles of 2- and 15-g *P. vannamei* and *P. monodon*

Species (weight; number of shrimp)	Average duration of moult stage in days ± SD (percentage of total cycle)					Duration of total cycle
	A	B	C	D1	D2	
P. vannamei (2.0 ± 0.3 g; $n = 36$)	0.5 ± 0.1 (10%)	0.5 ± 0.1 (11%)	0.6 ± 0.2 (12%)	1.7 ± 0.4 (35%)	1.5 ± 0.3 (32%)	4.8 ± 0.5[a*]
P. vannamei (14.8 ± 0.9 g; $n = 36$)	0.8 ± 0.3 (7.5%)	1.1 ± 0.5 (10%)	1.6 ± 0.5 (15%)	3.8 ± 0.8 (34.5%)	3.6 ± 0.7 (33%)	10.9 ± 1[b]
P. monodon (2.1 ± 0.5 g; $n = 12$)	0.5 ± 0.2 (8%)	1 ± 0.5 (16%)	1.3 ± 0.5 (20%)	1.8 ± 0.5 (28%)	1.9 ± 0.4 (30%)	6.4 ± 0.9[c]
P. monodon (15.2 ± 1.0 g; $n = 12$)	0.6 ± 0.1 (5%)	1.1 ± 0.3 (9%)	1.5 ± 0.4 (12%)	4.4 ± 0.7 (36%)	4.7 ± 0.6 (38%)	12.3 ± 0.6[d]

* Different subscripts indicate statistically significant differences

At 27 °C



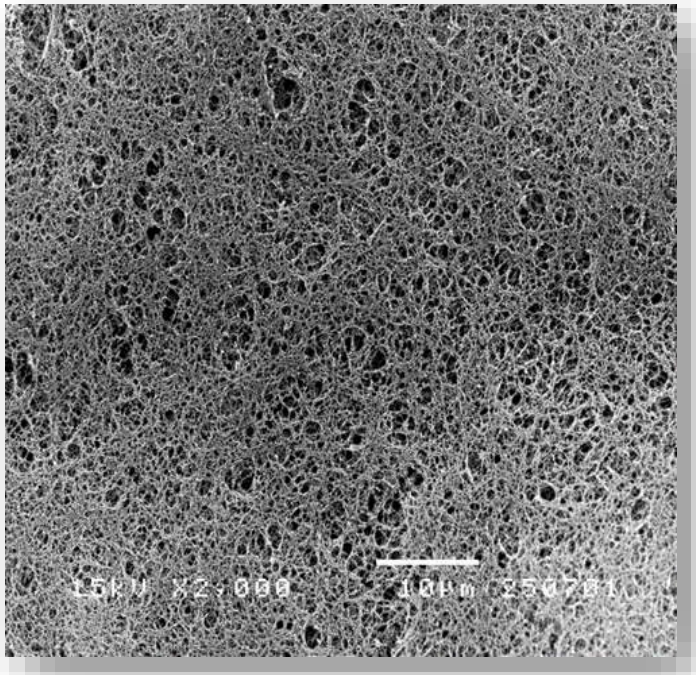
Chitin-based barrier immunity (The peritrophic membrane, PM)

is an ancient system

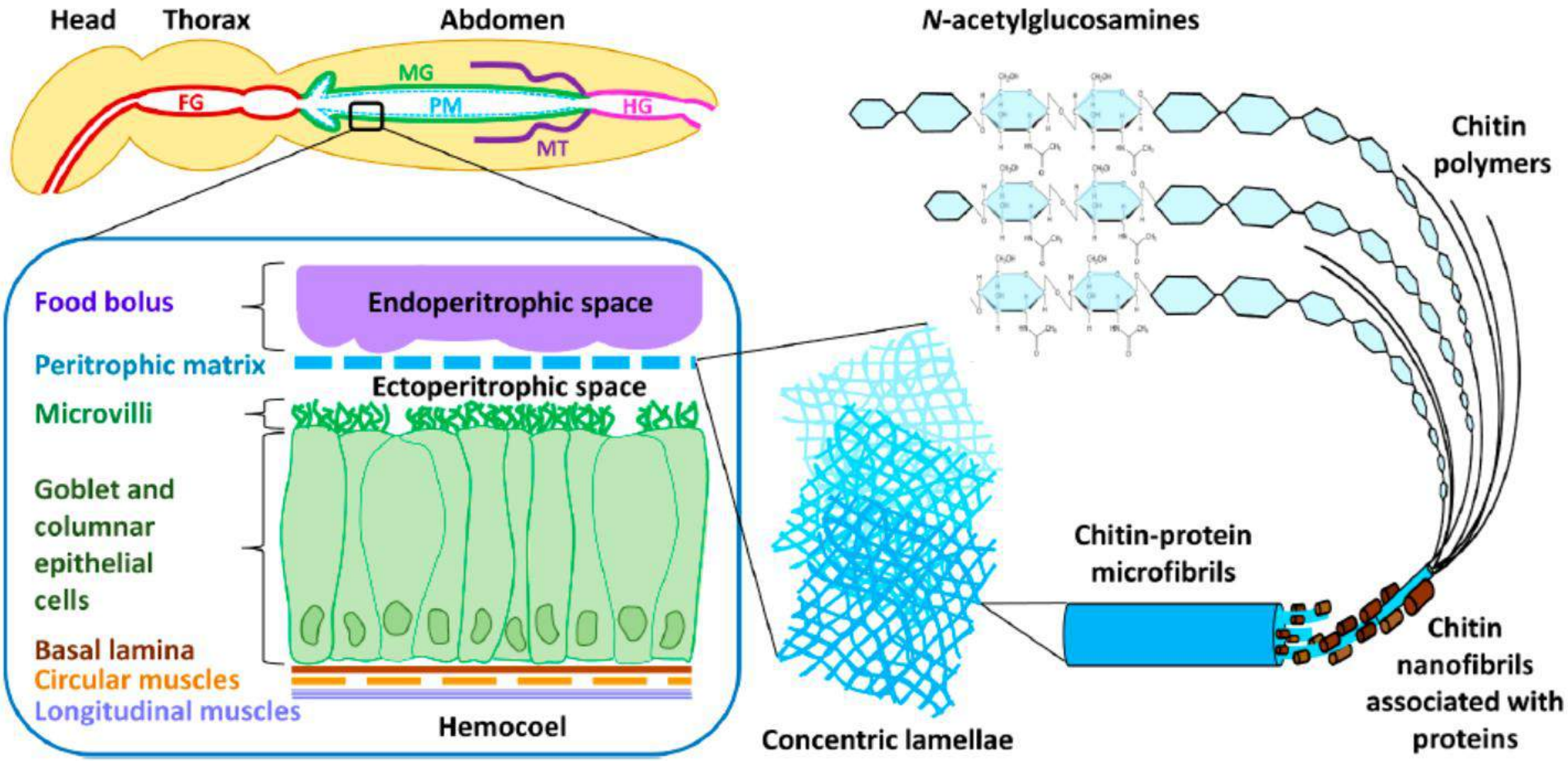
The PM serves as the first defense in the midgut

The PM is

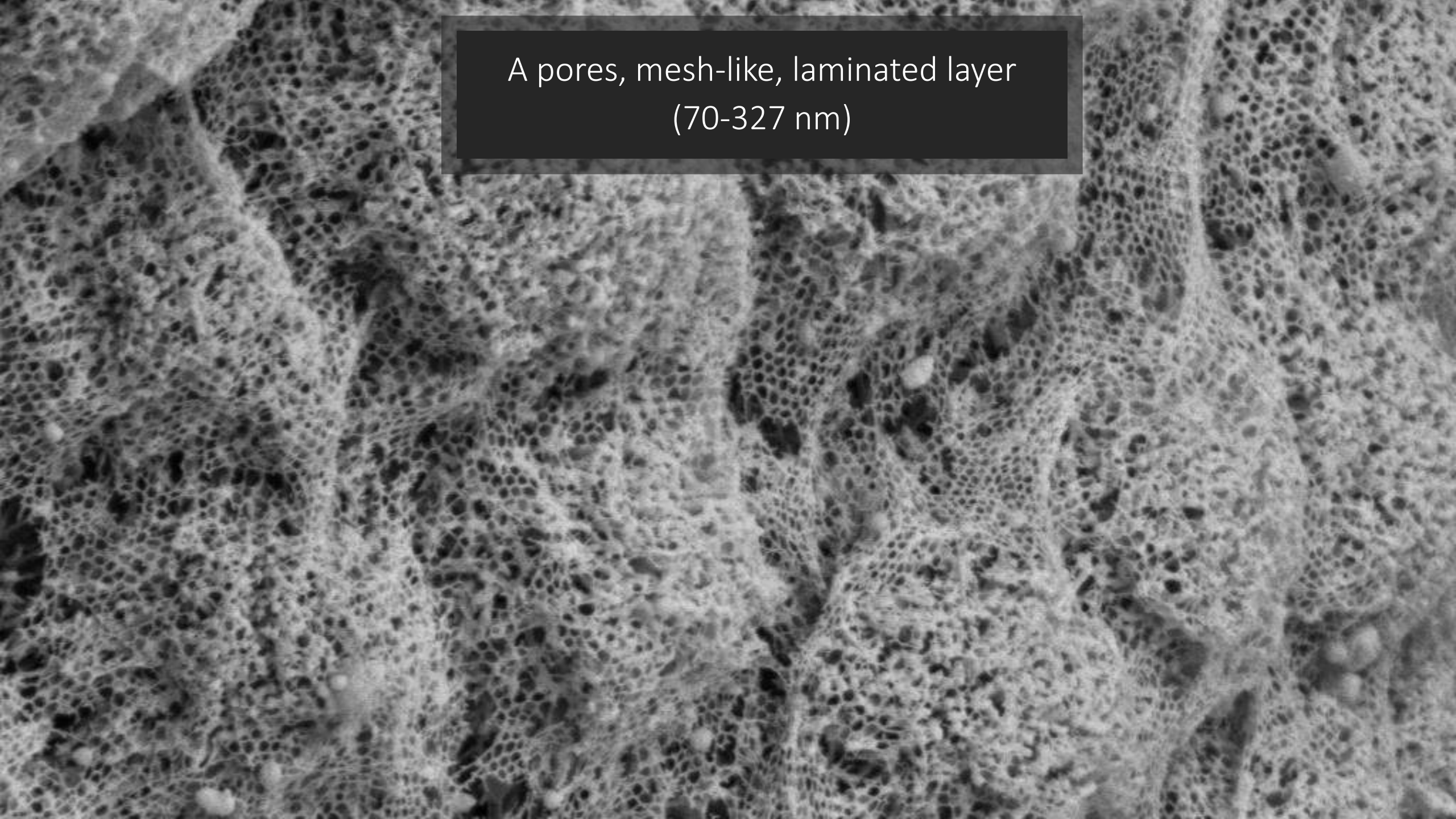
- an acellular matrix,
- semi-permeable biocomposite
- composed of chitin, protein and glycoprotein
- lining the invertebrate midgut

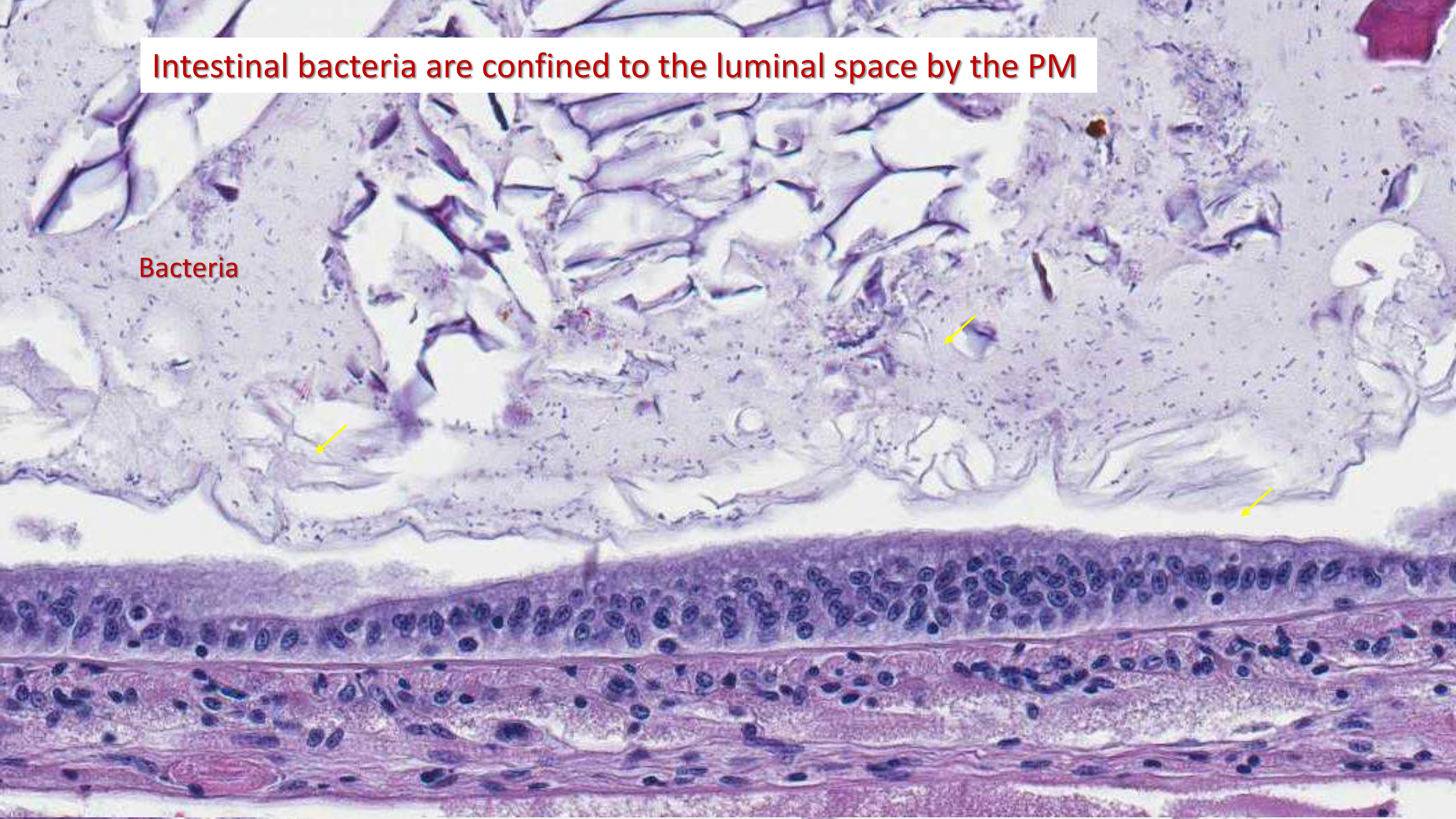


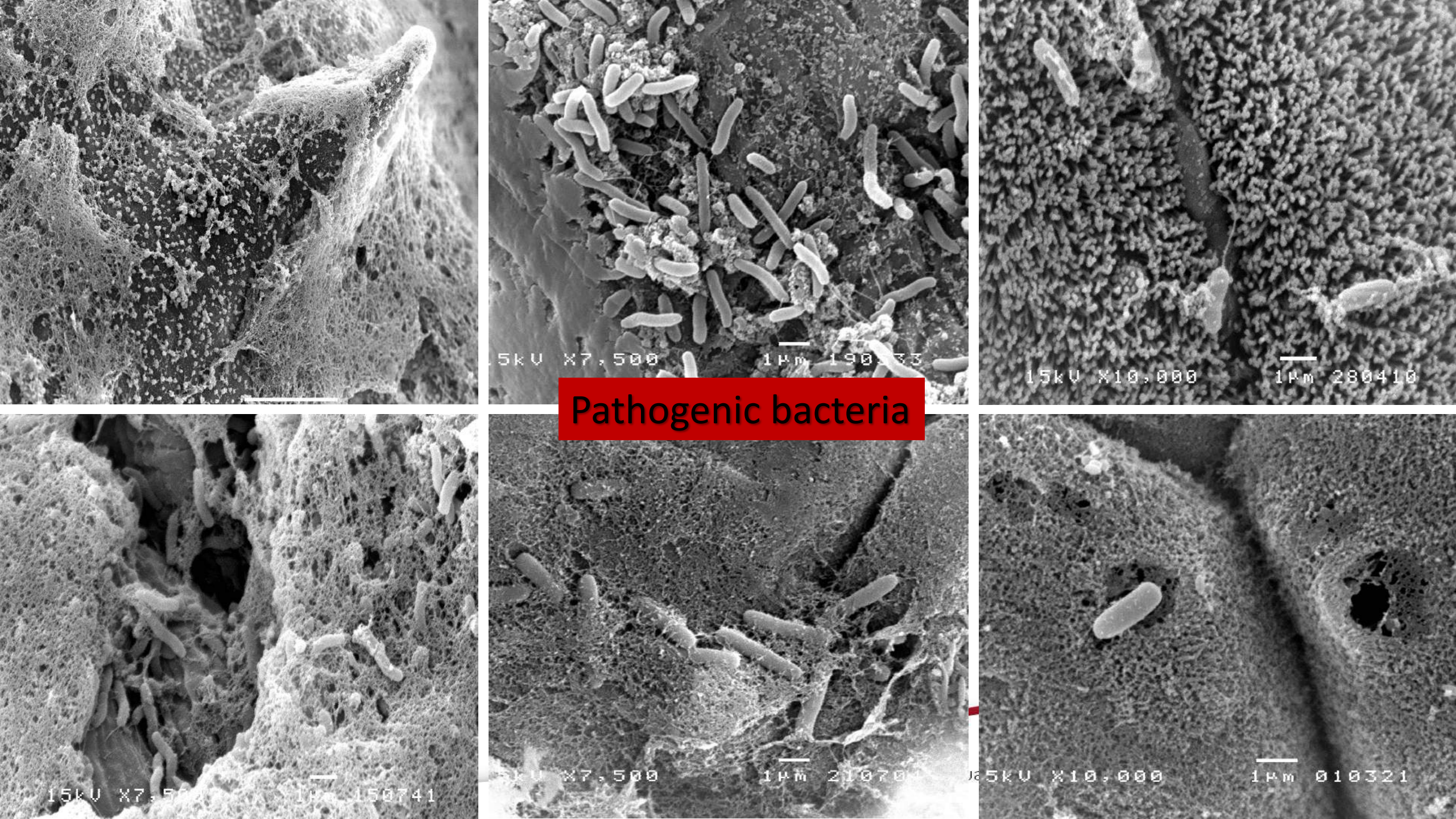
The PM is continuously biosynthesized, assembled, and degraded in response to feeding and development.



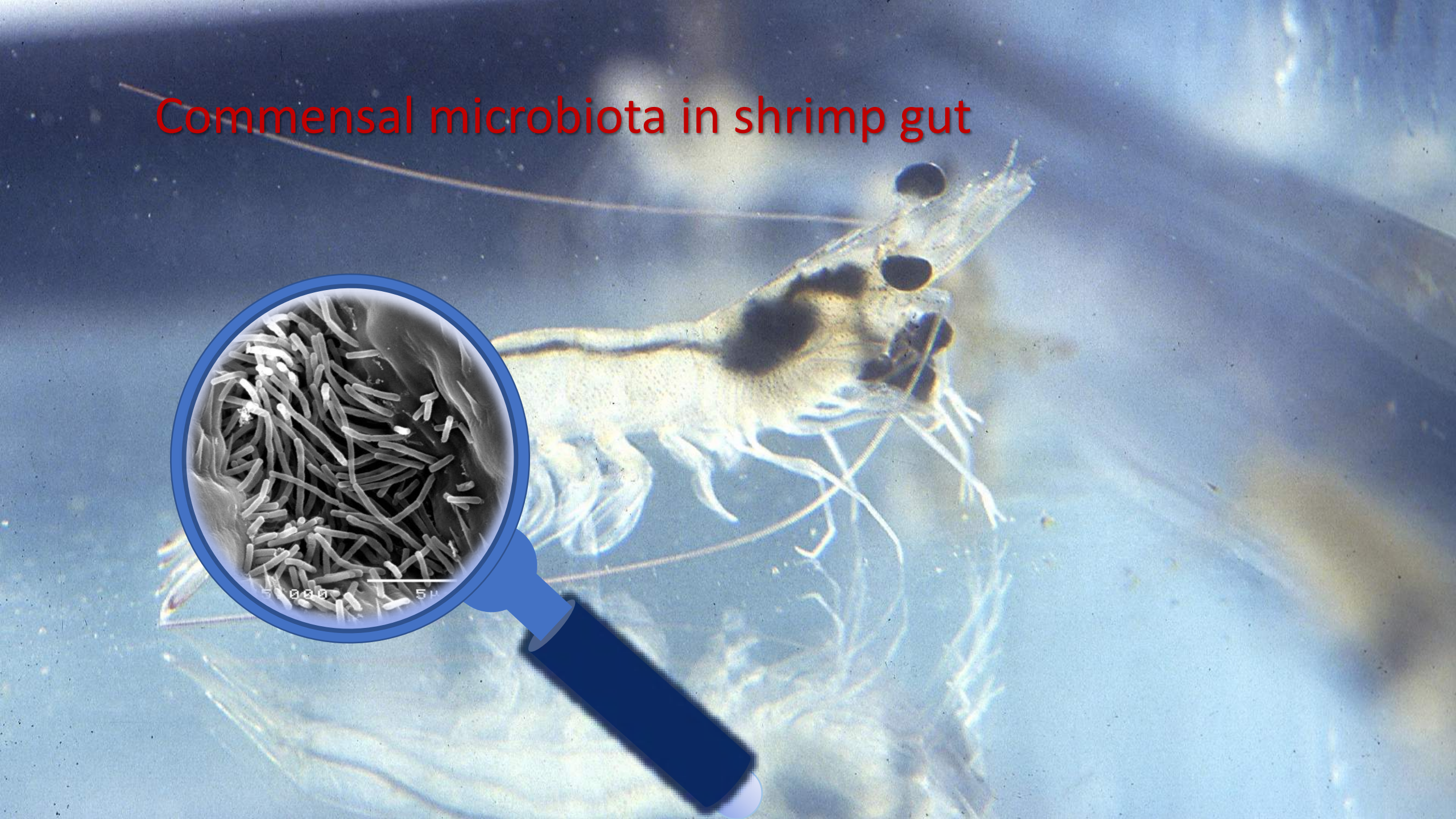
X. Liu, et al. Journal of Insect Physiology 114 (2019) 109–115


A pores, mesh-like, laminated layer
(70-327 nm)


Intestinal bacteria are confined to the luminal space by the PM
Bacteria

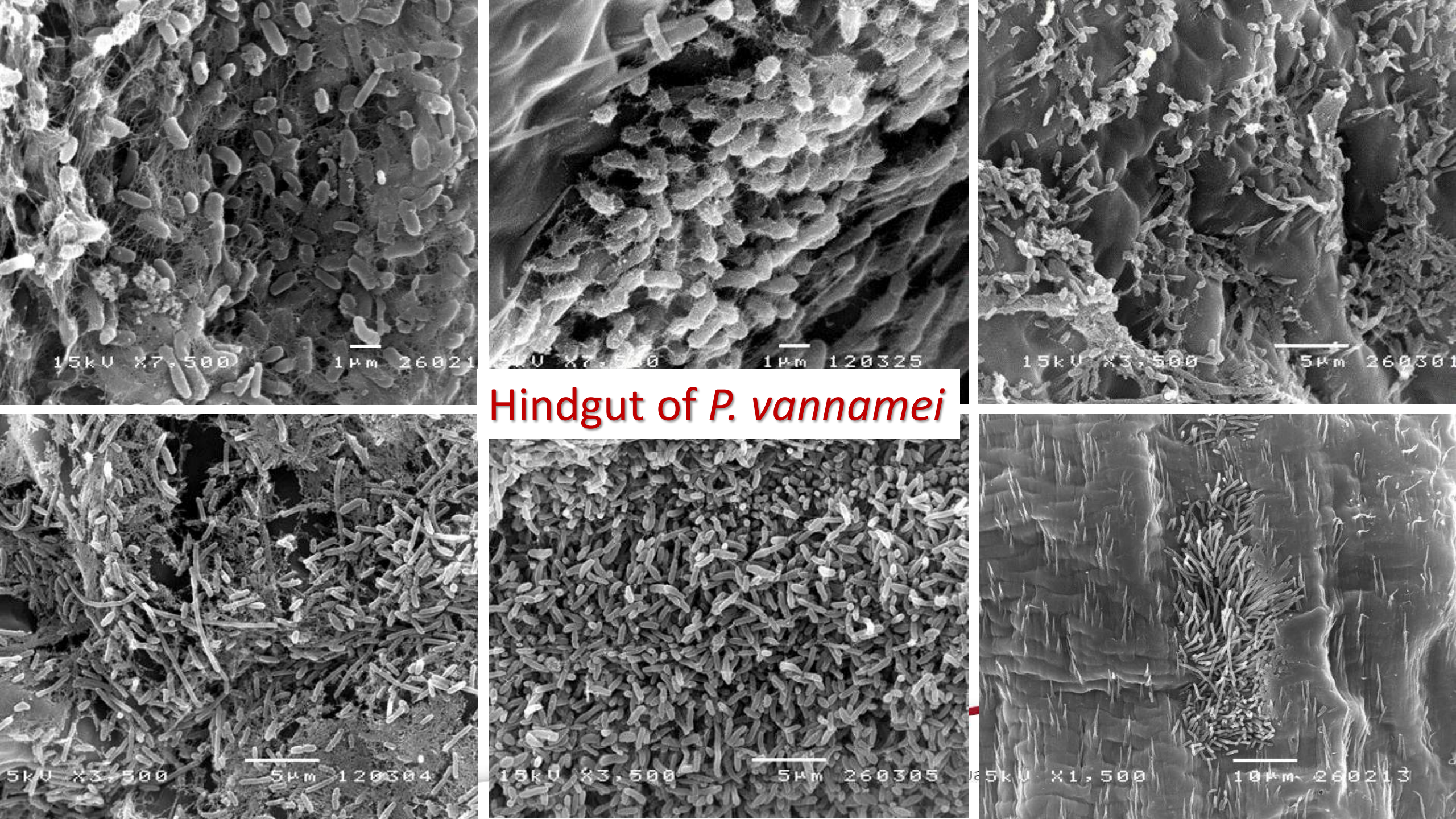

Pathogenic bacteria

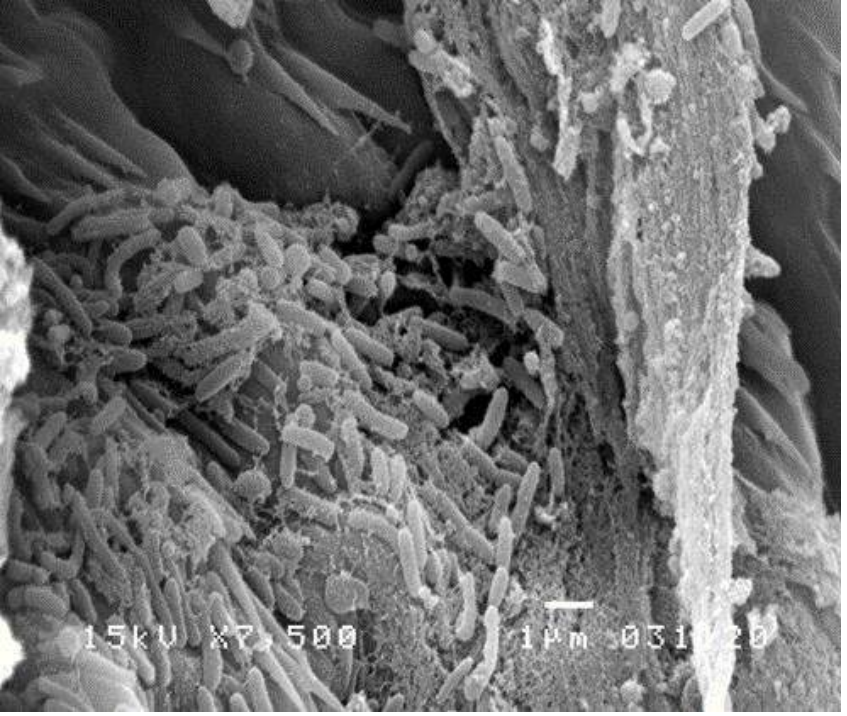
Commensal microbiota in shrimp gut

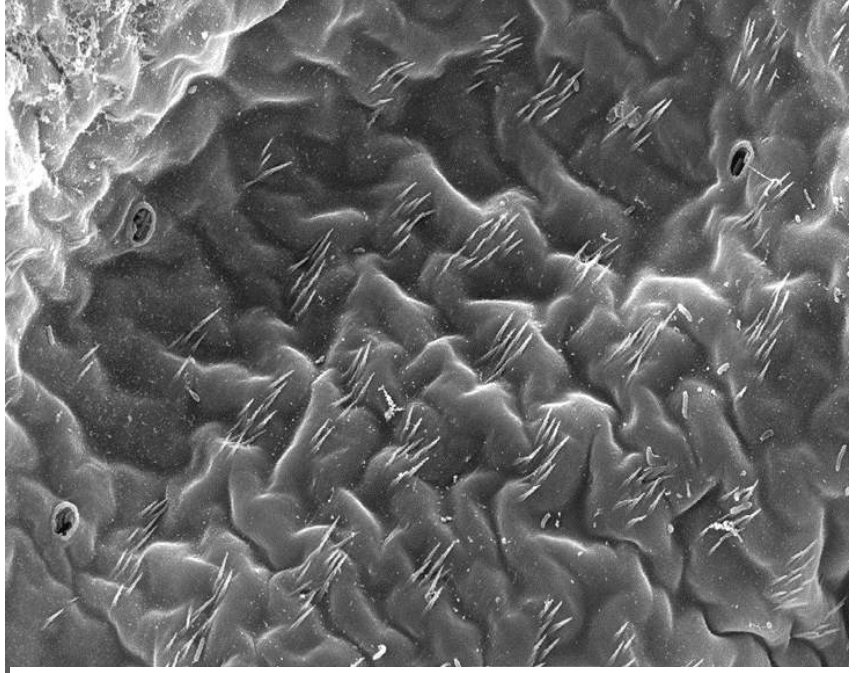


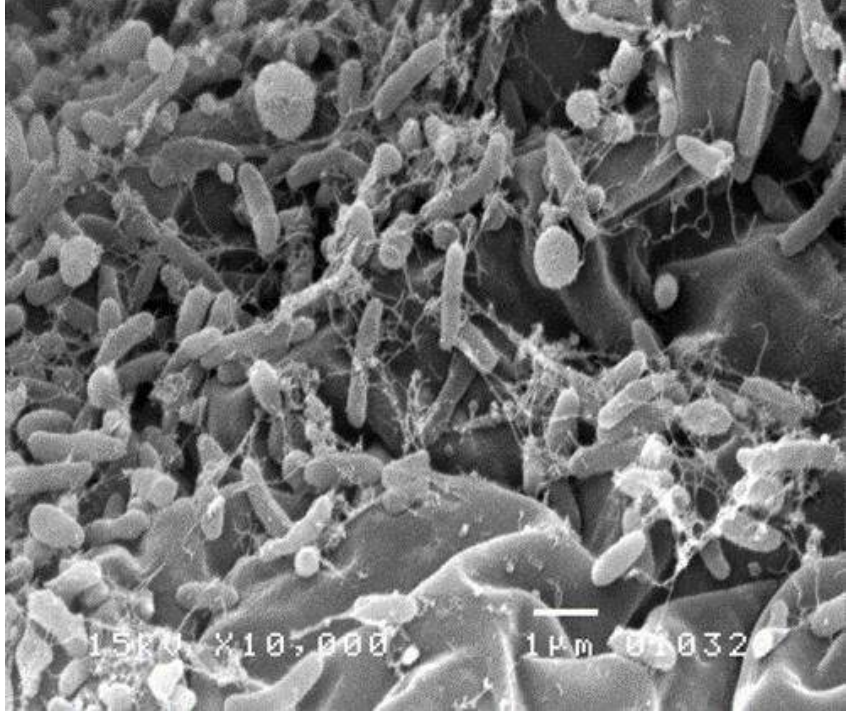
Where is commensal microbiota found?

? ? ? ? ? ?

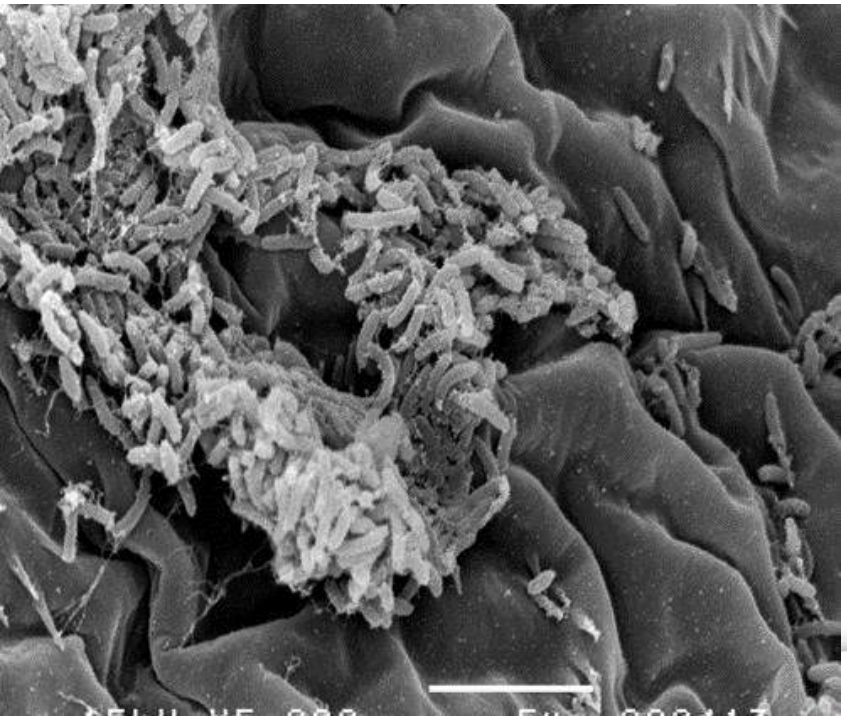

15kV X7,500 1µm 26021
1µm 120325
15kV X3,500 5µm 260301
Hindgut of P. vannamei
5kV X3,500 5µm 120304
15kV X3,500 5µm 260305
5kV X1,500 10µm 260213

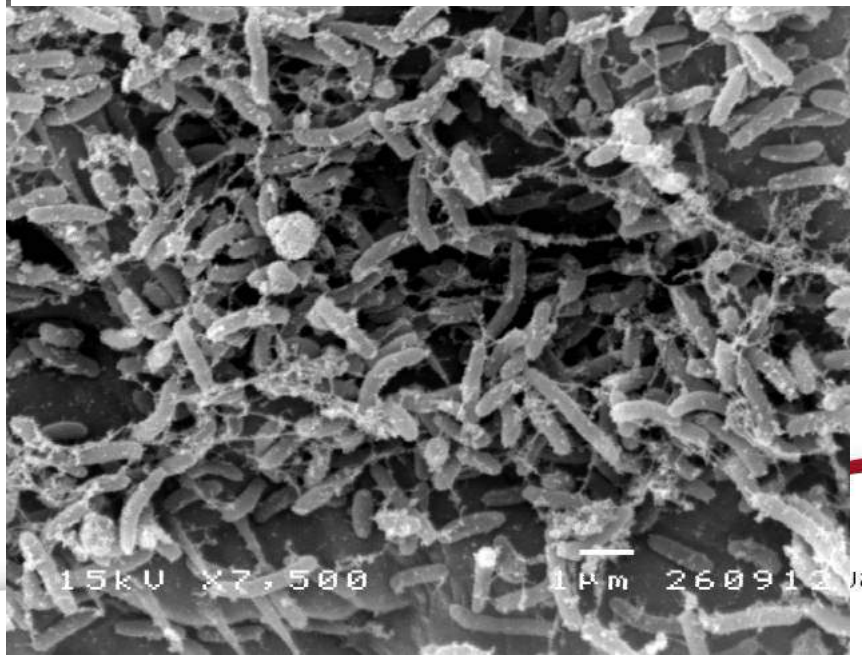


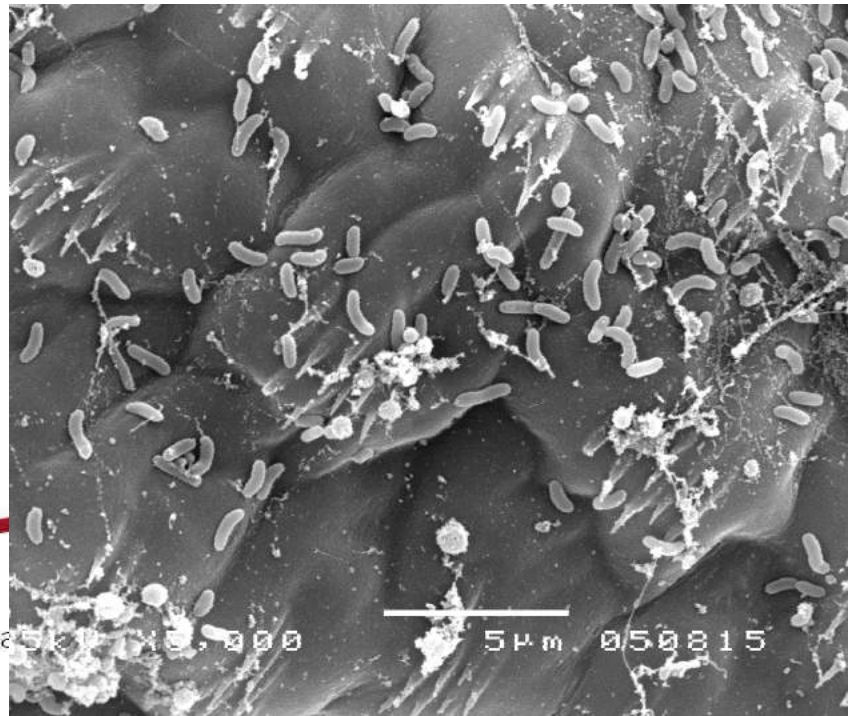




Hindgut of *P. monodon*

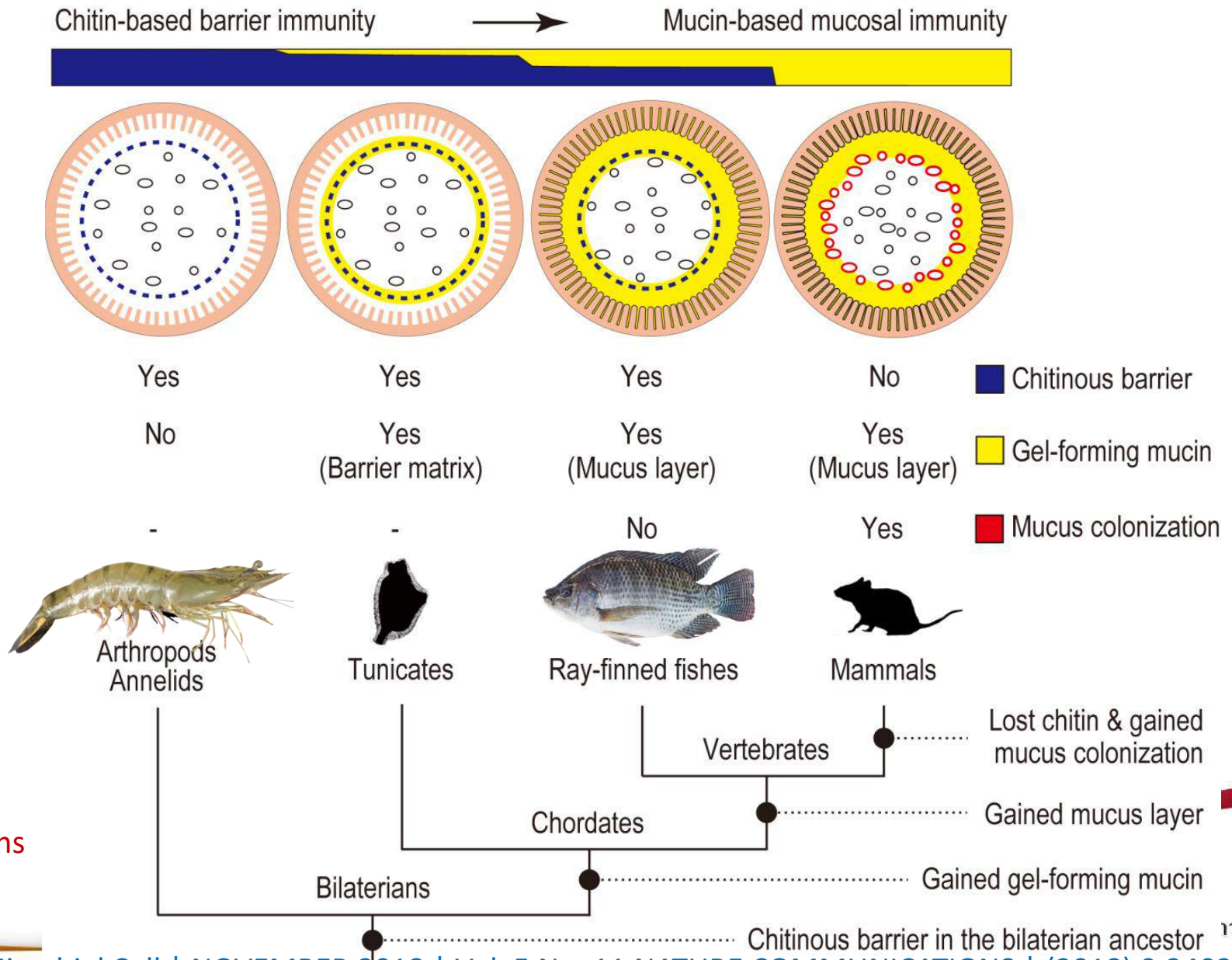






Is there a presence of commensal bacteria in the midgut?

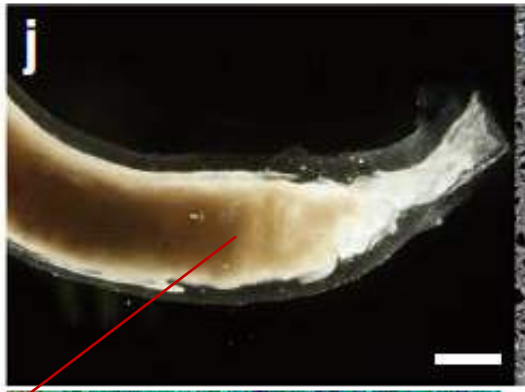
Intestinal barrier structure

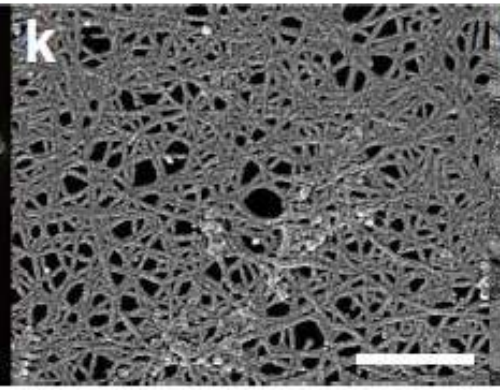


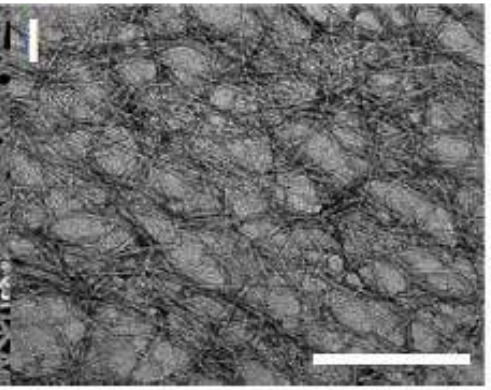
Nakashima K. 2018.
Nature communications

Ray-finned fish

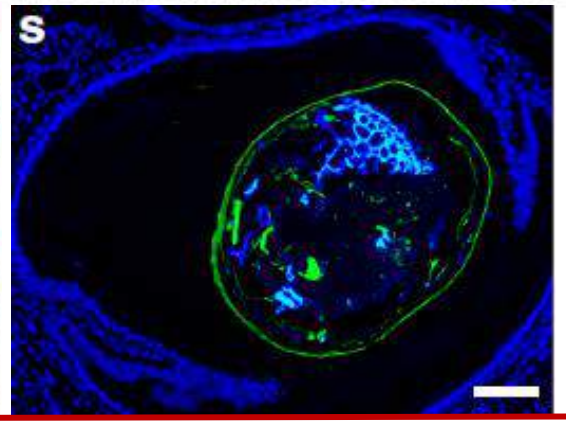
Vertebrate



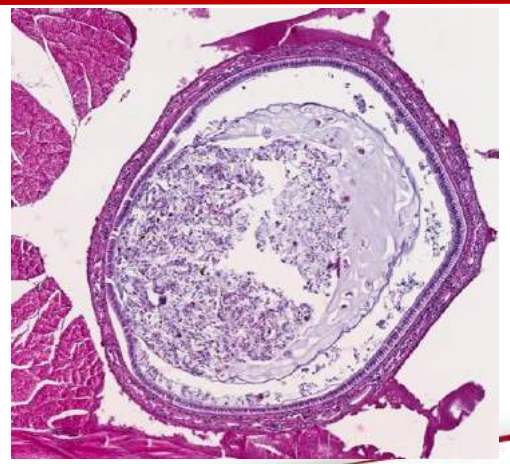


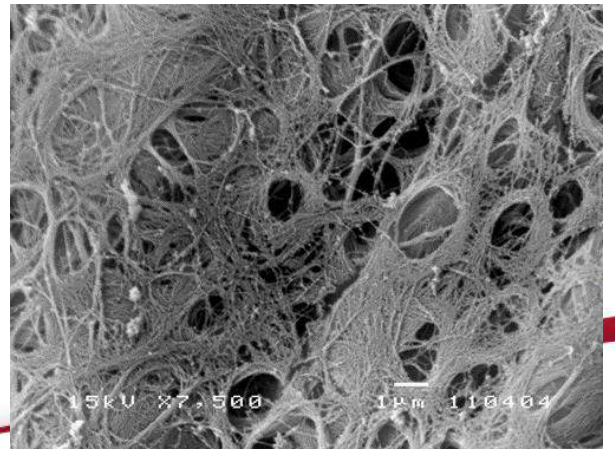


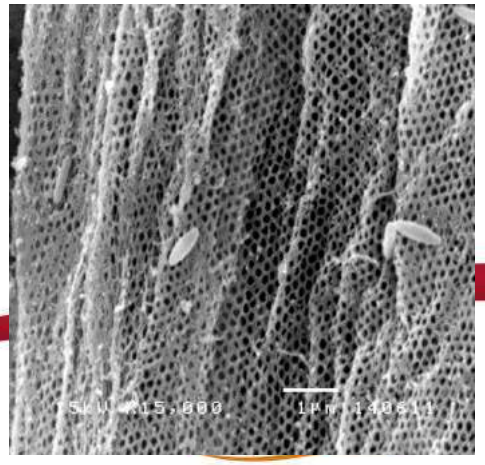
Envelope membrane



Invertebrate

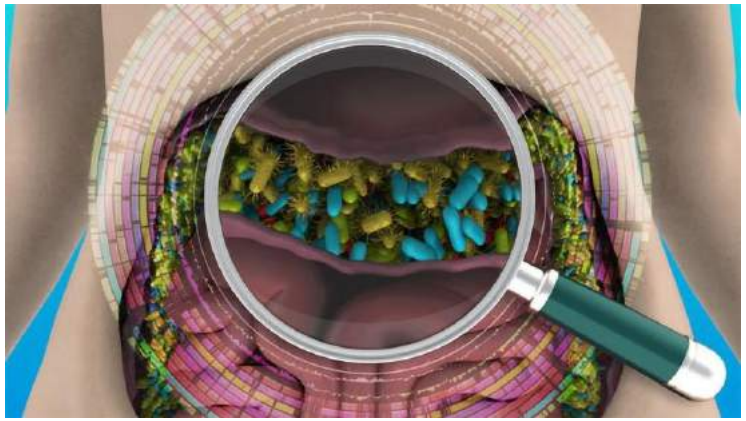


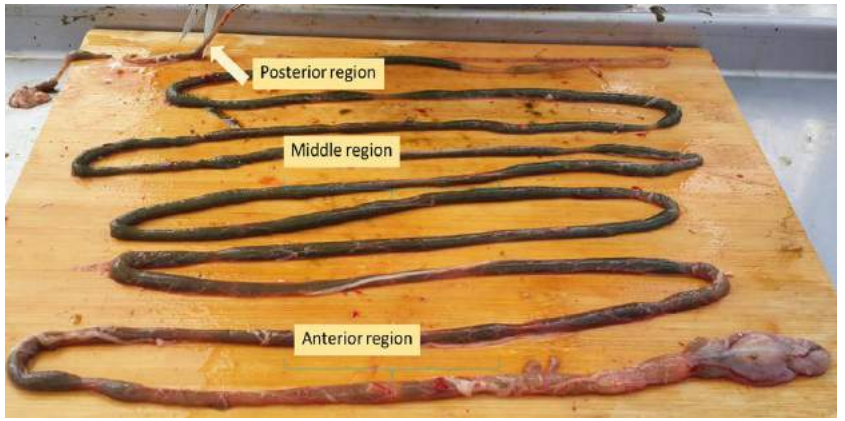




Gastrointestinal transit time: Human Fish and Shrimp

Human





About 1 hour

About 10 hours-several days

About 10-14 hours ?

Gastrointestinal transit time is the interval time between ingestion of food and its elimination as feces.

Conclusion

	Teleost Fish	Shrimp
Biological class	Vertebrate	Invertebrate
Immune system	Innate and adaptive	Innate
Barrier immunity in the gut	Chitin-based and Mucin-based barrier	Chitin-based barrier
Commensal microbiota	Yes	?

Although shrimp and fish have distinct traits, their first line of defense is similar.

Thank you