Mucosal immunity in bivalves

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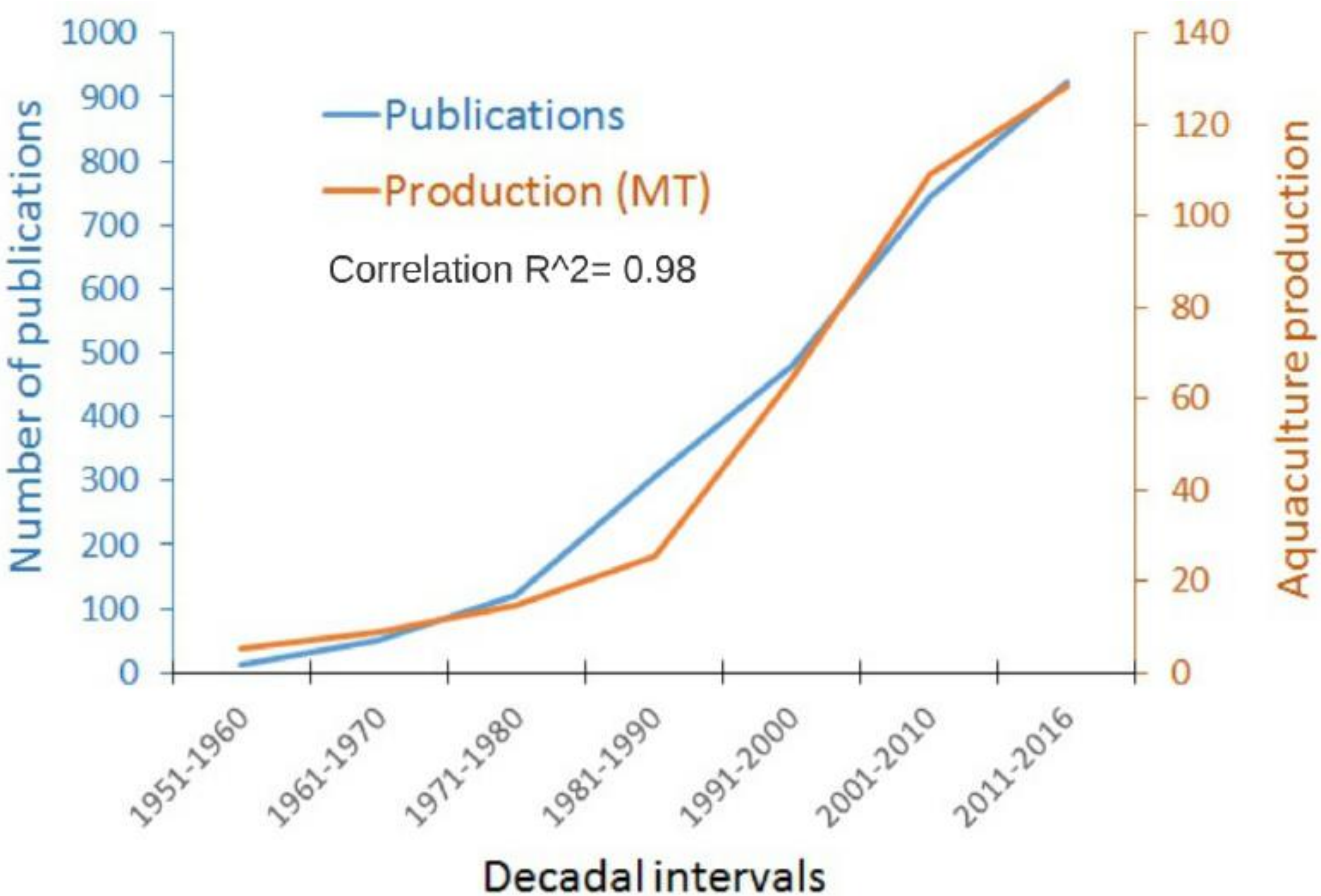
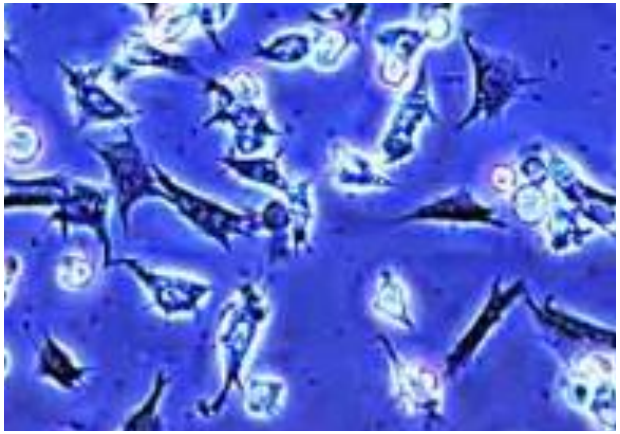
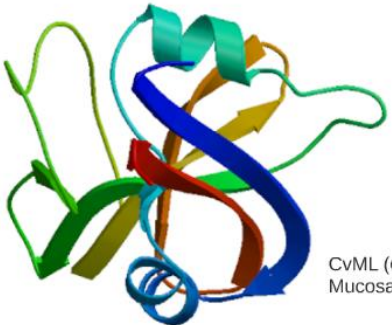
Rapid growth in immunity research parallels production growth

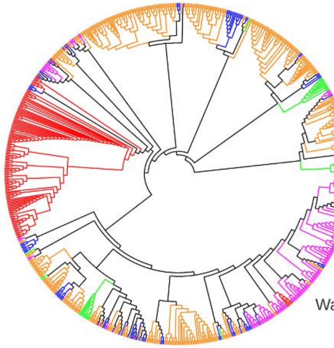


Significant progress

- Cellular and humoral processes of innate immunity
- Diversity of immune recognition factors (e.g. hypervariable immune proteins)
- Immune priming
- Role of the microbiome







Pending questions:

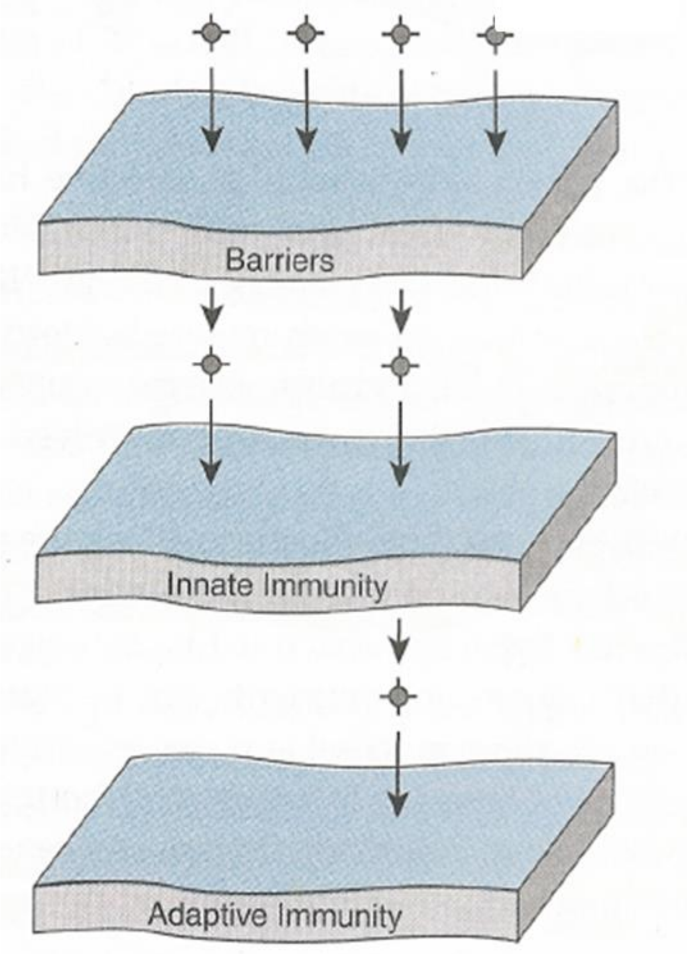
- What are the biological bases for resistance to infections

Immunity is provided by a multi-layered system

Only one particular site in animals encompasses all of these barriers:

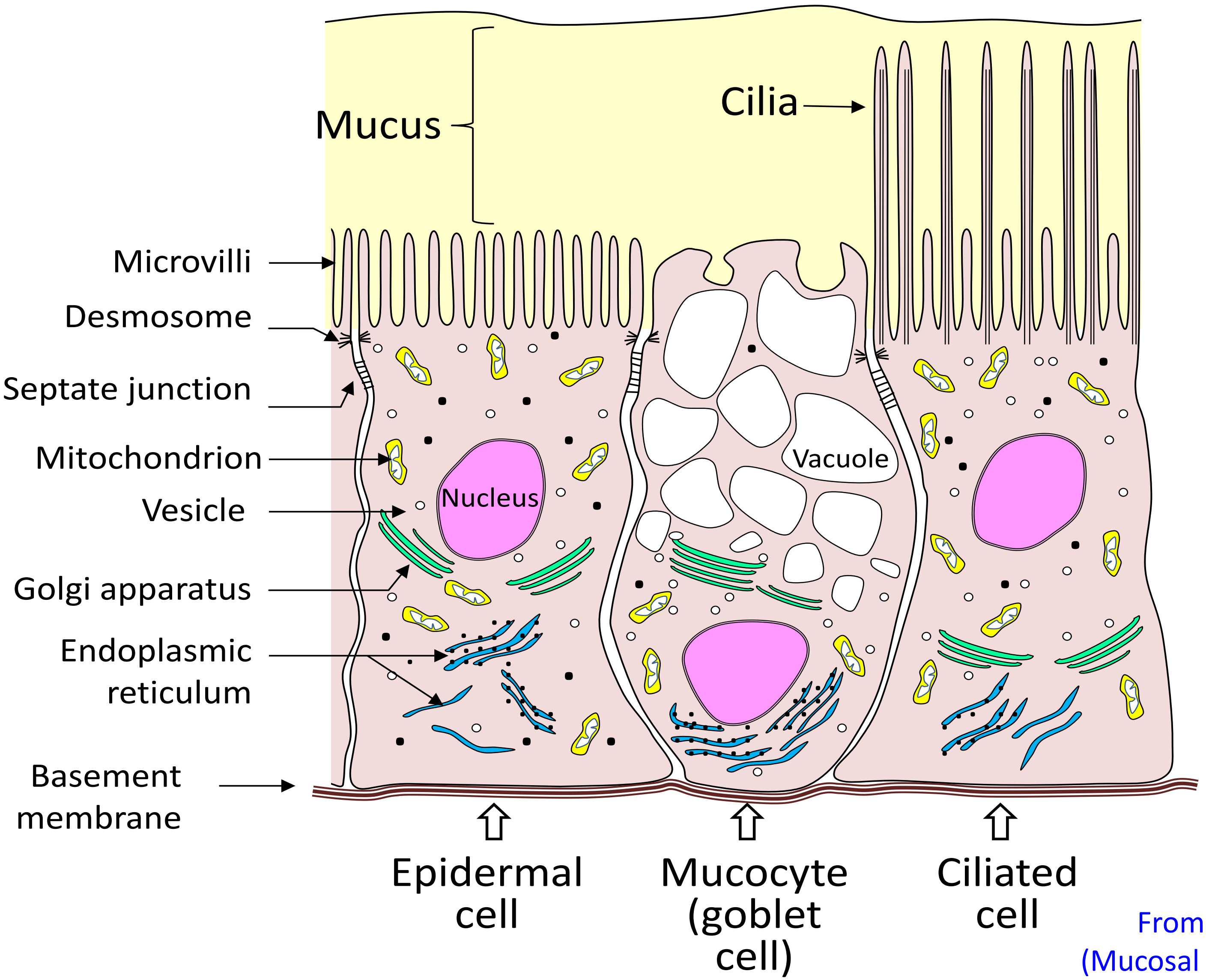
MUCOSAL INTERFACES

Invading microorganisms



Mucus: a great physical and biological barrier

Abundant mucosal secretions are produced by molluscan epithelial cells and subepithelial glands



- Can exceed 15% of energy gained from food (over 50% in some gastropods)
- Barrier to diffusion
- Selective ion transport

From Allam and Pales Espinosa, 2015
(Mucosal health in aquaculture, AP book)

Main mucosal epithelia in bivalve mollusks

Water processing organs

Major role of mucus in the transport of particles captured by the gills

Limited information on the role of bivalve mucus in immunity

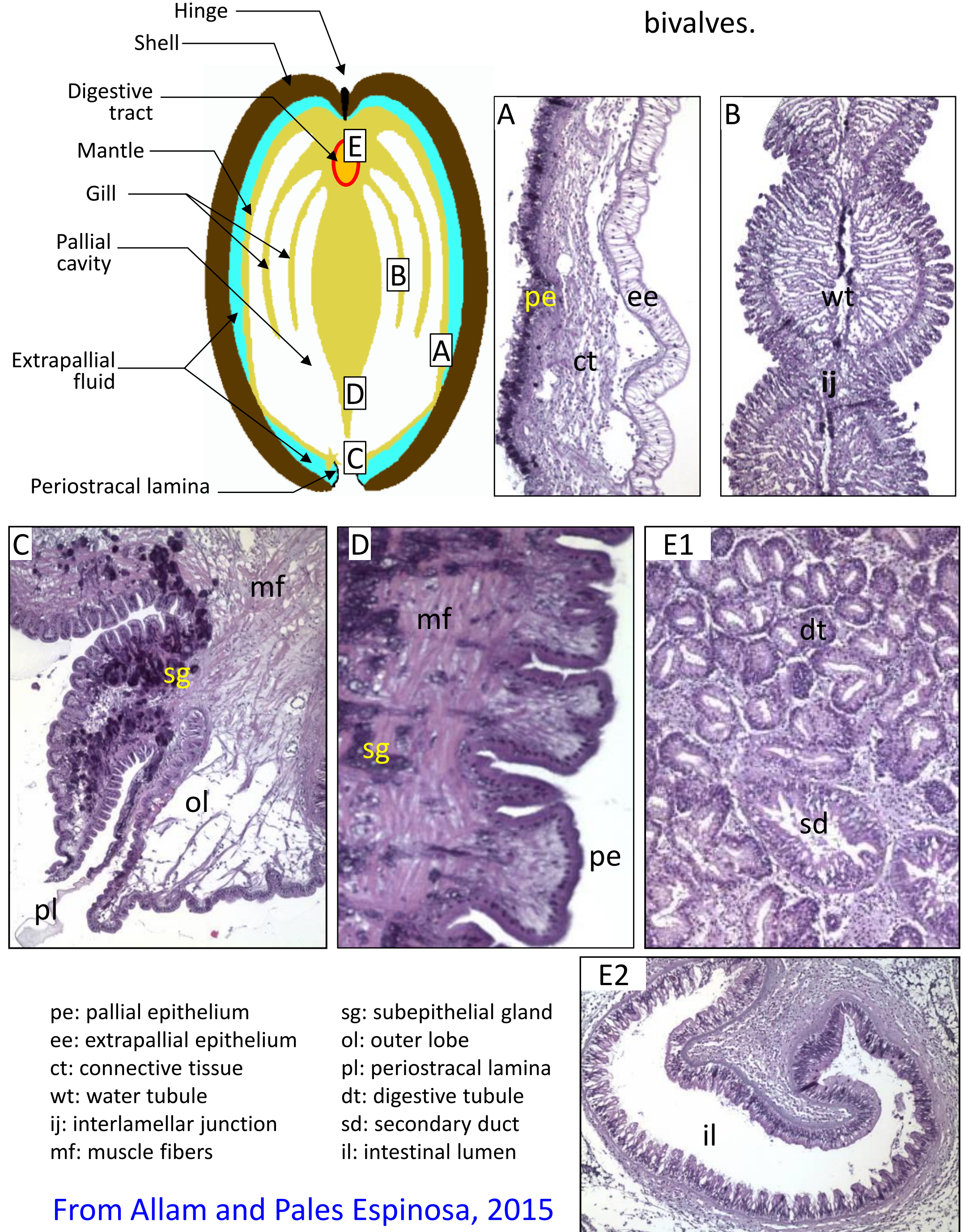
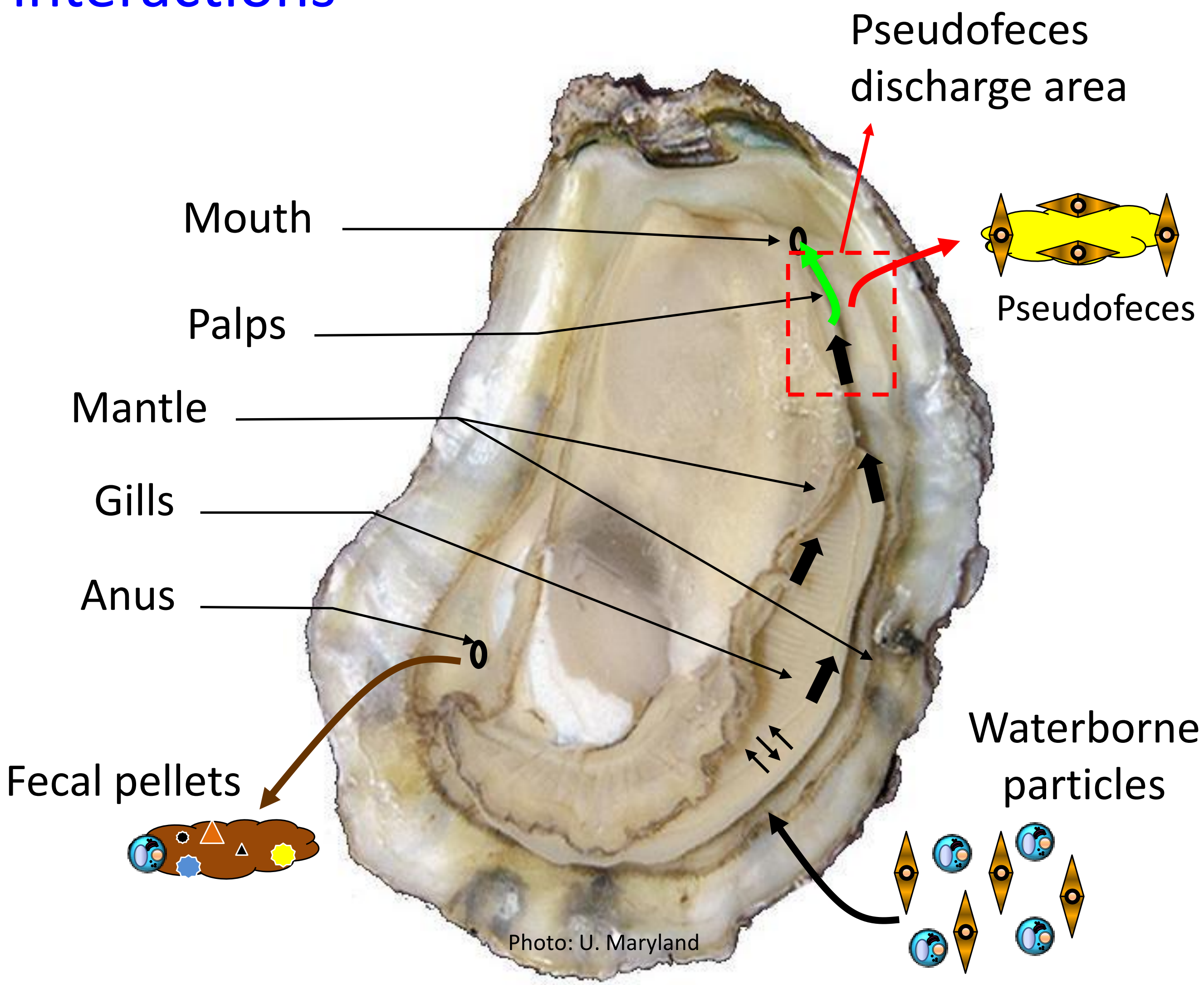
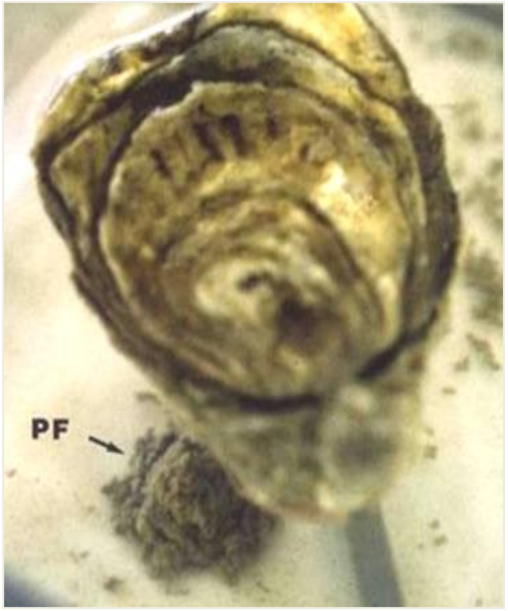


Figure 3. Main mucosal epithelia in bivalves.

Bivalve pallial surfaces: hotspots for host-microbe interactions

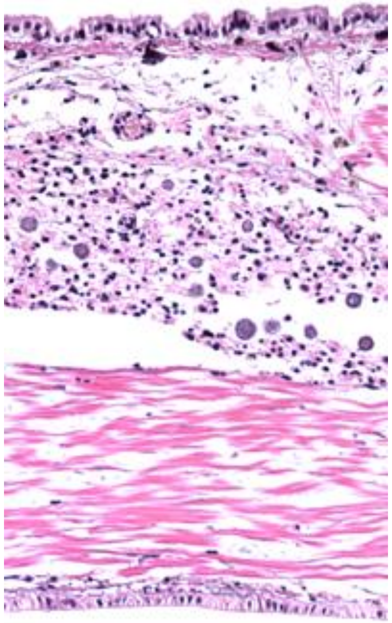




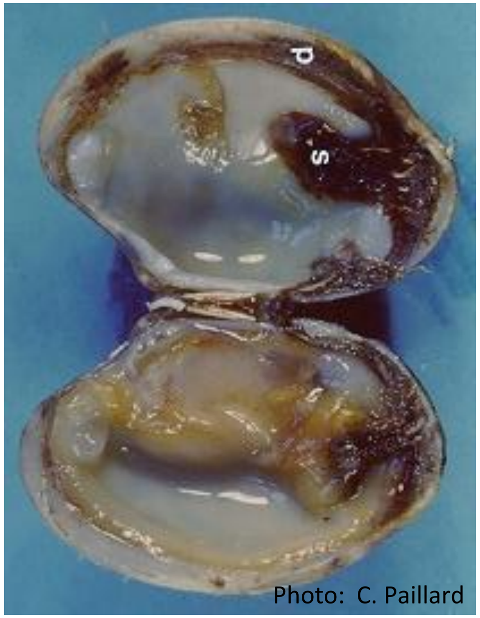
Oysters filter >10 L h^{-1} g^{-1} dry tissue = over 25,000,000 microbes/second (considering a 1 g oyster and 10^7 microbes ml^{-1} seawater)

Pathogen acquisition mechanisms in marine bivalves

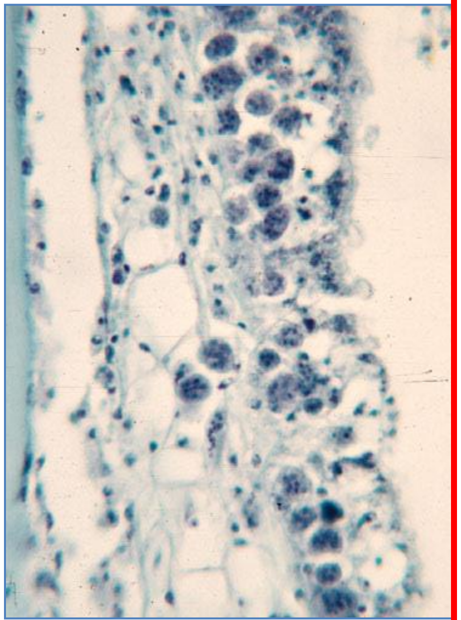
- Digestive tract (via mechanical and chemical activities) is an excellent barrier to infectious agents and most fatal infections affecting bivalve mollusks are initiated in pallial organs (mantle and gills)



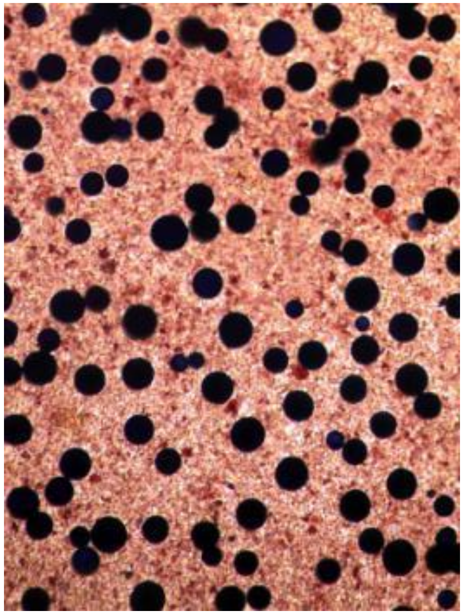
QPX (clam mantle)



Vibrio tapetis (clam mantle)



MSX (oyster gill)



Perkinsus marinus (oyster mantle)

Contents lists available at SciVerse ScienceDirect
Journal of Invertebrate Pathology
journal homepage: www.elsevier.com/locate/jip

Early host-pathogen interactions in marine bivalves: Evidence that the alveolate parasite *Perkinsus marinus* infects through the oyster mantle during rejection of pseudofeces

Bassem Allam[a,*], Wade E. Carden[a,1], J. Evan Ward[b], Gina Ralph[b], Sarah Winnicki[a,2], Emmanuelle Pales Espinosa[a]

Vol. 104: 237–247, 2013 doi: 10.3354/dao02599 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published June 13

Early host–pathogen interactions in a marine bivalve: *Crassostrea virginica* pallial mucus modulates *Perkinsus marinus* growth and virulence

Emmanuelle Pales Espinosa, Sarah Winnicki, Bassem Allam*

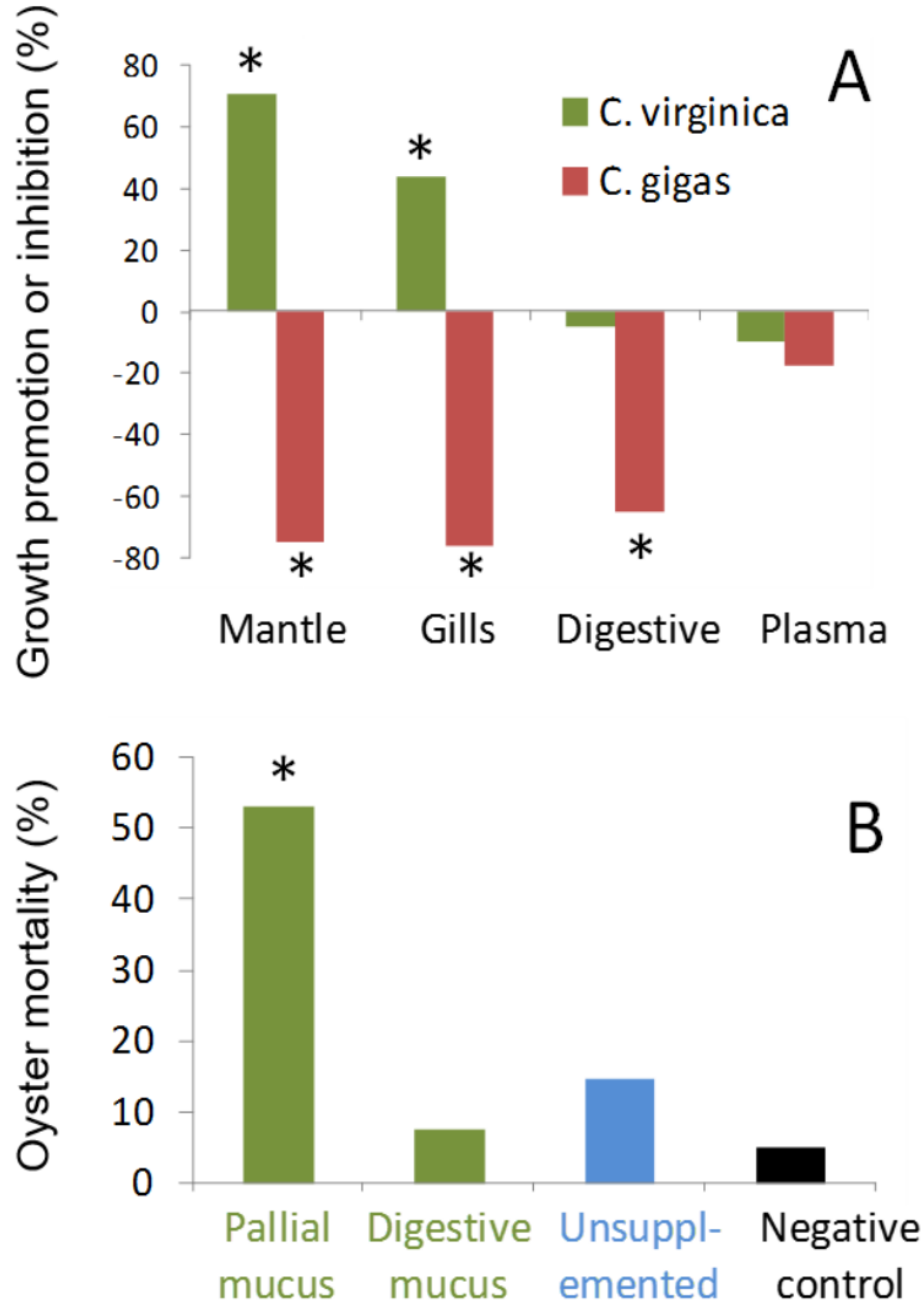
School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York 11794-5000, USA

Contents lists available at ScienceDirect
International Journal for Parasitology
journal homepage: www.elsevier.com/locate/ijpara

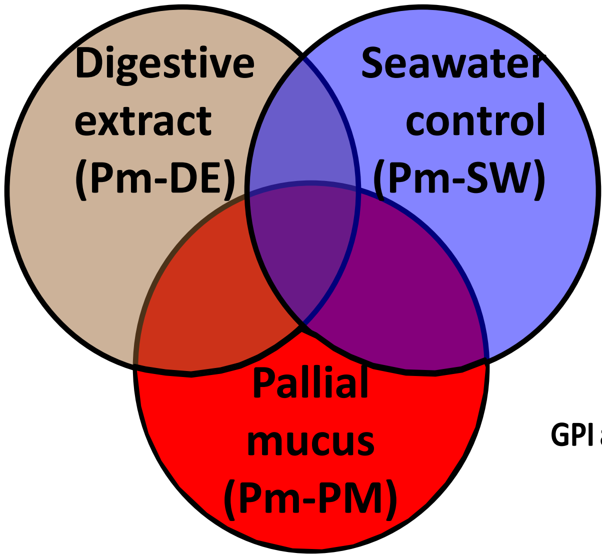
Pallial mucus of the oyster *Crassostrea virginica* regulates the expression of putative virulence genes of its pathogen *Perkinsus marinus* ☆

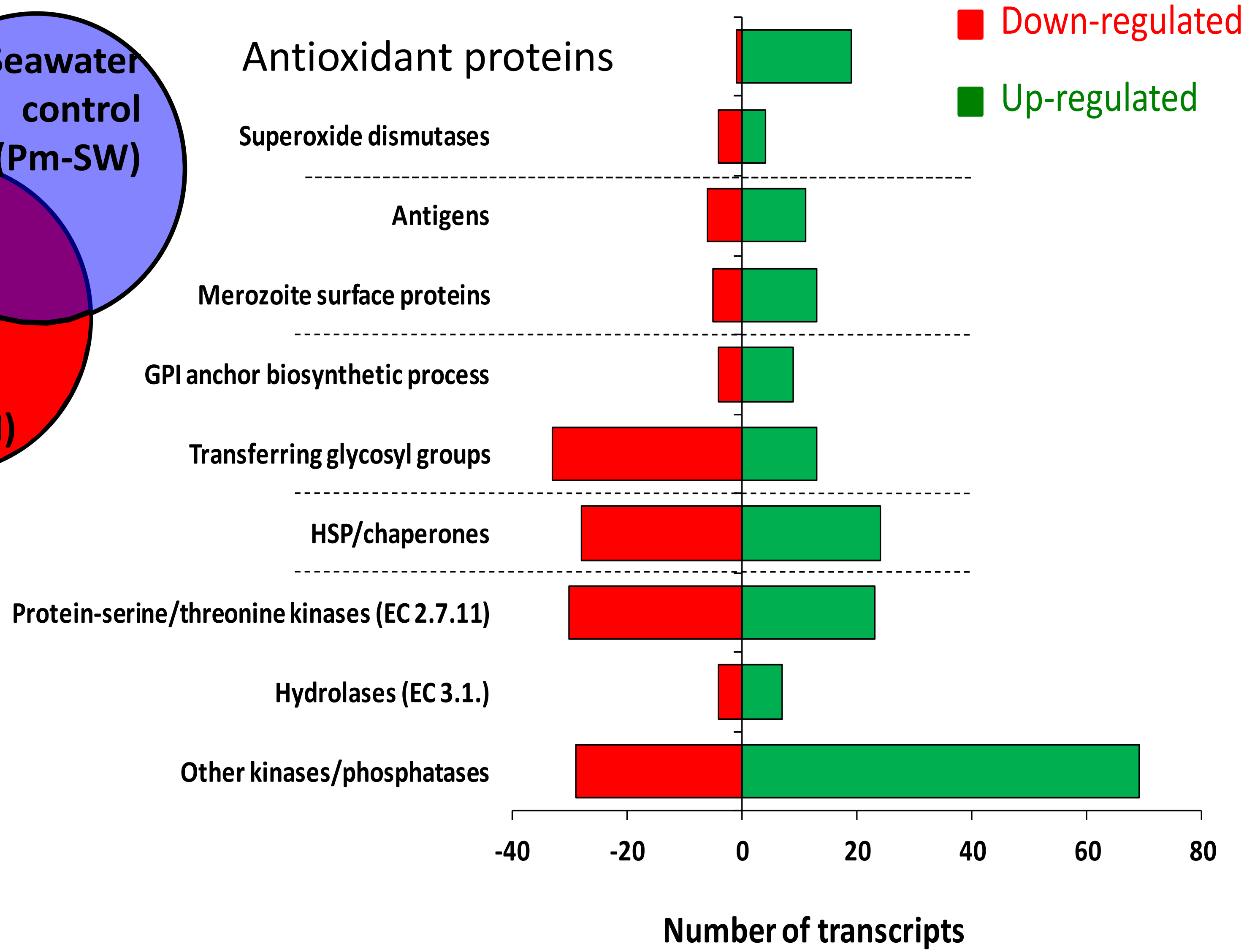
Emmanuelle Pales Espinosa[a], Erwan Corre[b], Bassem Allam[a,*]

Significant regulation of virulence-related genes (RNASeq) in *P. marinus* following exposure to mucus



Results: RNASeq





Pales Espinosa et al. (2014). Int. J. Parasitology

RNASeq: some transcripts involved in interactions with host

Function	Up-regulated	Down-regulated
Pro-apoptotic	**14 transcripts**: Apoptosis inducing factors LPS-induced TNF-α associated factor Macrophage migration inhibitory	**6 transcripts:** Apoptosis-inducing factor Programmed cell death
Anti-apoptotic	**11 transcripts:** 14-3-3 proteins Apoptosis antagonizing transcription factor Fas apoptotic inhibitory	**1 transcript:** polyubiquitin

- Manipulation of host cell apoptosis is an important trait of *P. marinus* infection in oyster (work by Sunila et al., Sokolova et al.)
- Parasite cyclophylins neutralize host antimicrobial peptides

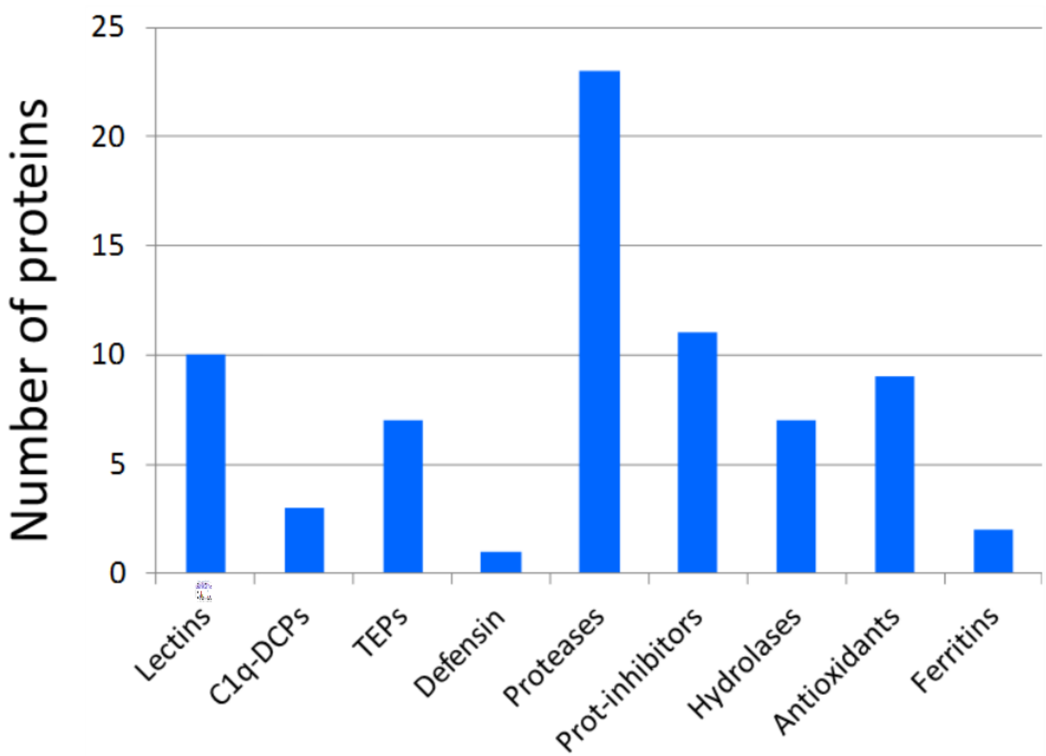
Pales Espinosa et al. (2014). Int. J. Parasitology

What immune factors are present in mucus? Proteomic analysis (LC-MS/MS) of oyster mucus

ELSEVIER
Contents lists available at ScienceDirect
Journal of Proteomics
journal homepage: www.elsevier.com/locate/jprot

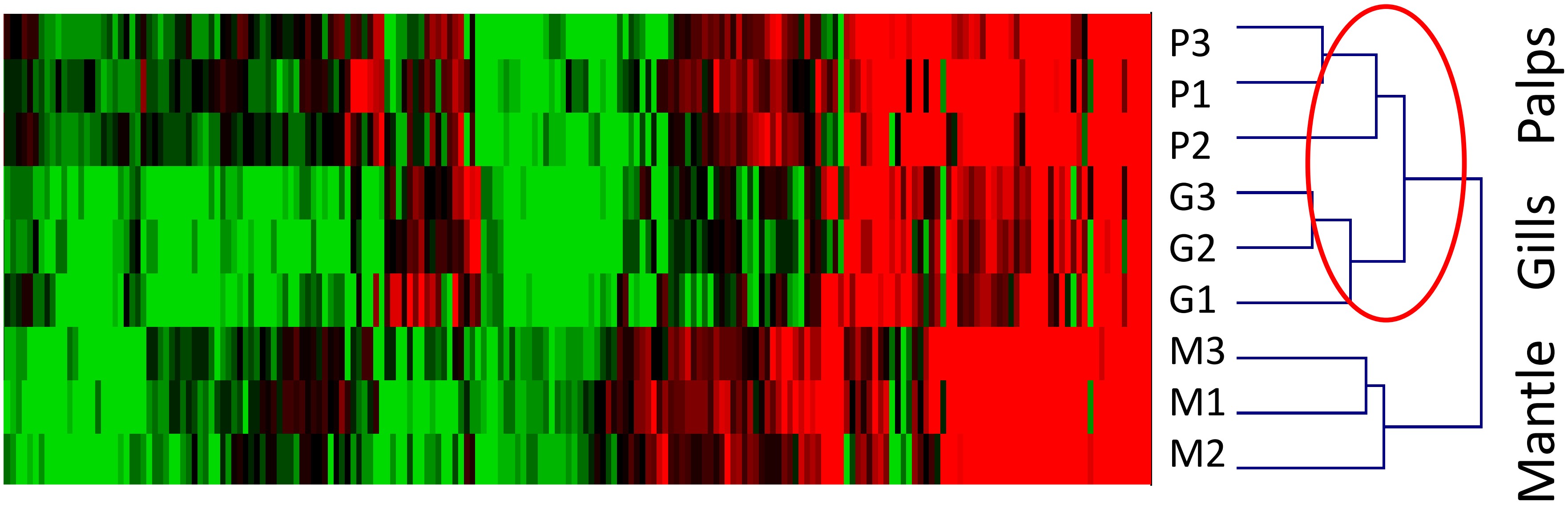
Proteomic characterization of mucosal secretions in the eastern oyster, *Crassostrea virginica*

Emmanuelle Pales Espinosa [a,*], Antonius Koller [b], Bassem Allam [a]



What immune factors are present in mucus?

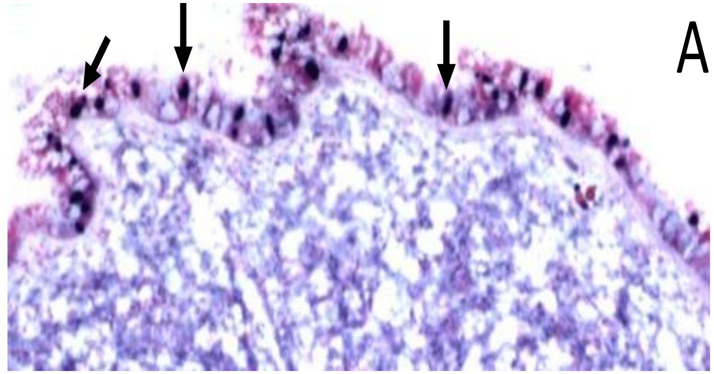
- Some proteins are differentially expressed in mucus derived from different organs



Mucus very rich in antimicrobial proteins including those involved in microbial binding (19 proteins: peptidoglycan-recognition protein, lectins) and killing (80 proteins: lysozyme, defensins)

Protein	Signal P	GenBank	eValue
C-type lectin-1 (CvML)	Signal P	BAF75353	1.97E-78
Galectin 2 (CvGal)	No	ABG75998	0
Macrophage mannose receptor 1	Extracellular/ Signal P	EKC26386	7.54E-64
Complement c1q-like proteins	Signal P	EKC25476	0
Thioester-containing proteins (TEPs)	Extracellular	EKC33672	0
Defensin	No	P85008	1.67E-18
Lysozyme 1	Extracellular/ Signal P	P83673	3.24E-116
Serine protease inhibitor (CvSI-2)	Extracellular/ Signal P	B9A8D7	1.01E-41

Molecular characterization of several mucosal lectins in bivalves



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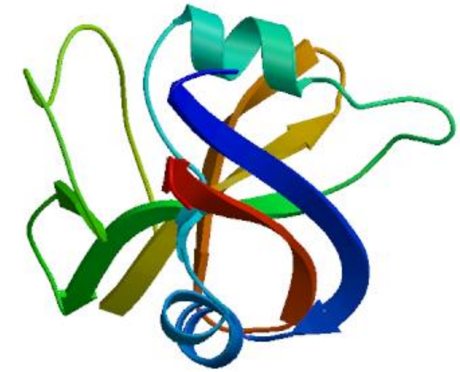
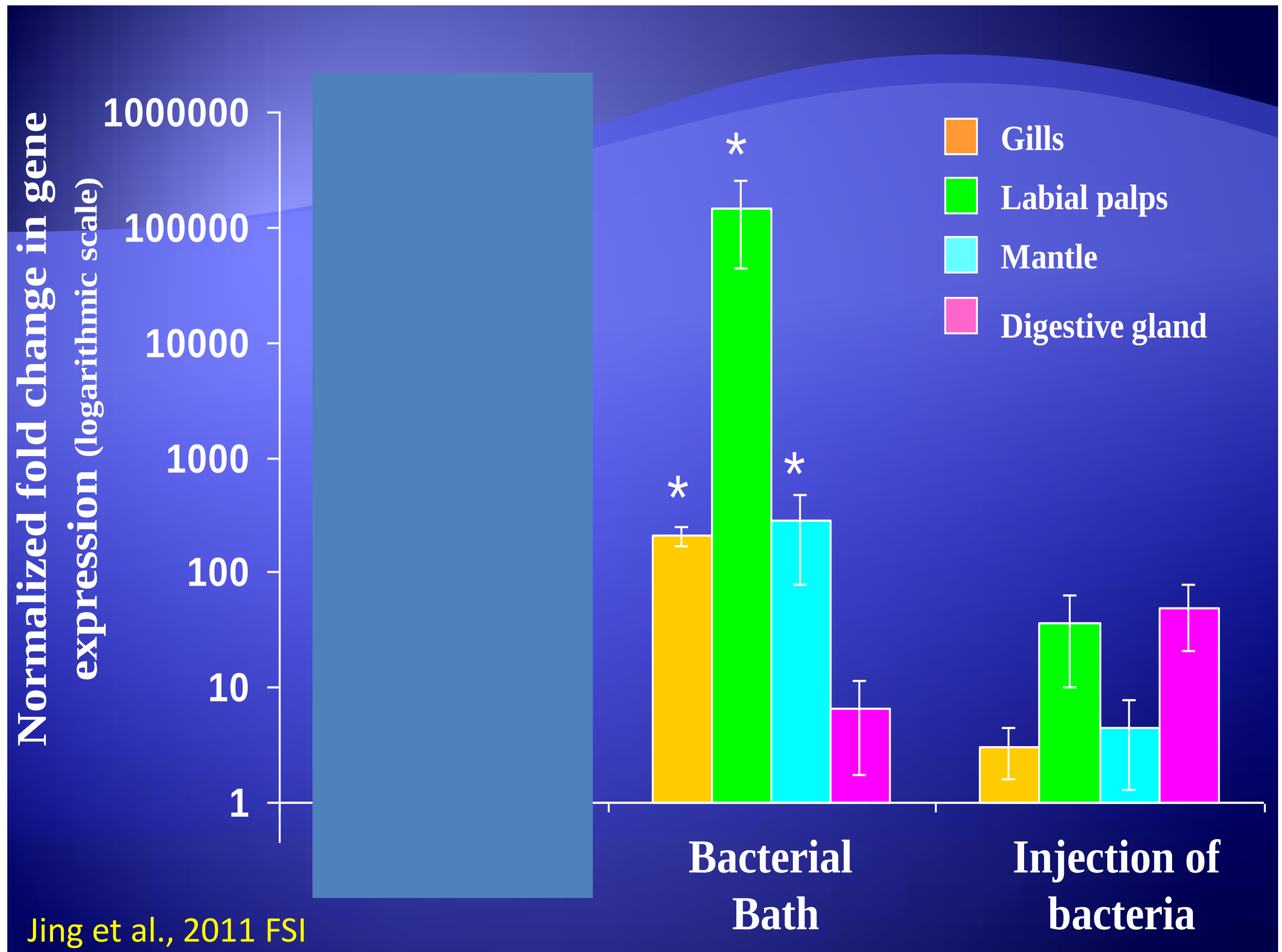
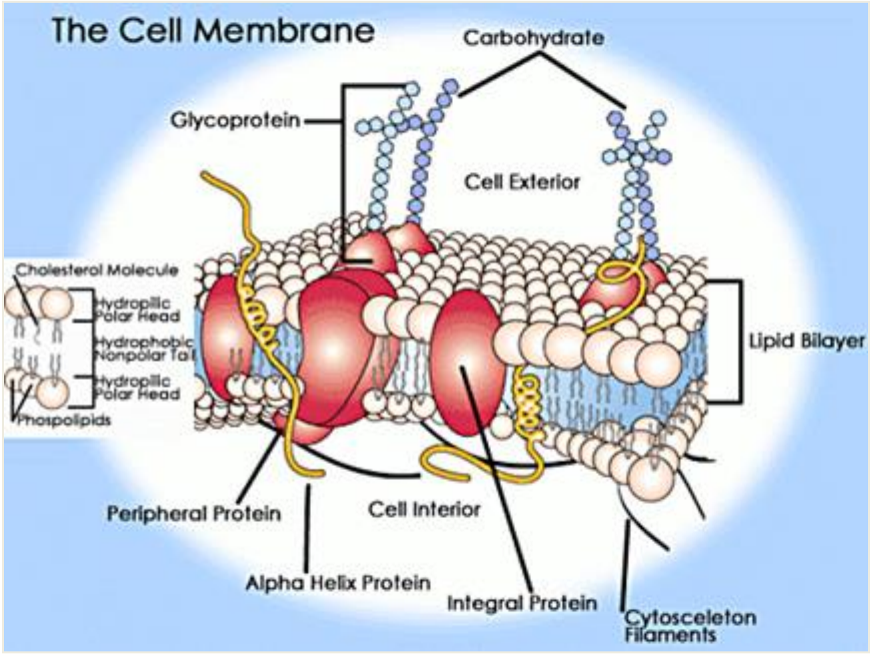


CvML (*Crassostrea virginica* mucocyte lectin) transcript levels (qPCR) 72 hrs after exposure to different treatments



Some of these mucosal lectins interact with carbohydrates associated with microbial (microalgae) cell surface triggering their capture

Pales Espinosa et al., Biol Bull 2009, 2010a, 2010b; Jing et al., FSI 2011; Pales Espinosa and Allam, Mar Biol 2013



Mar Biol (2016) 163:40
DOI 10.1007/s00227-016-2815-0

CrossMark

ORIGINAL PAPER

Modeling food choice in the two suspension-feeding bivalves, *Crassostrea virginica* and *Mytilus edulis*

Emmanuelle Pales Espinosa[1] · Robert M. Cerrato[1] · Gary H. Wikfors[2] · Bassem Allam[1]

Oysters and mussels preferentially ingest microalgae having mannose residues on their cell surface

© 2018. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2018) 221, jeb174094. doi:10.1242/jeb.174094

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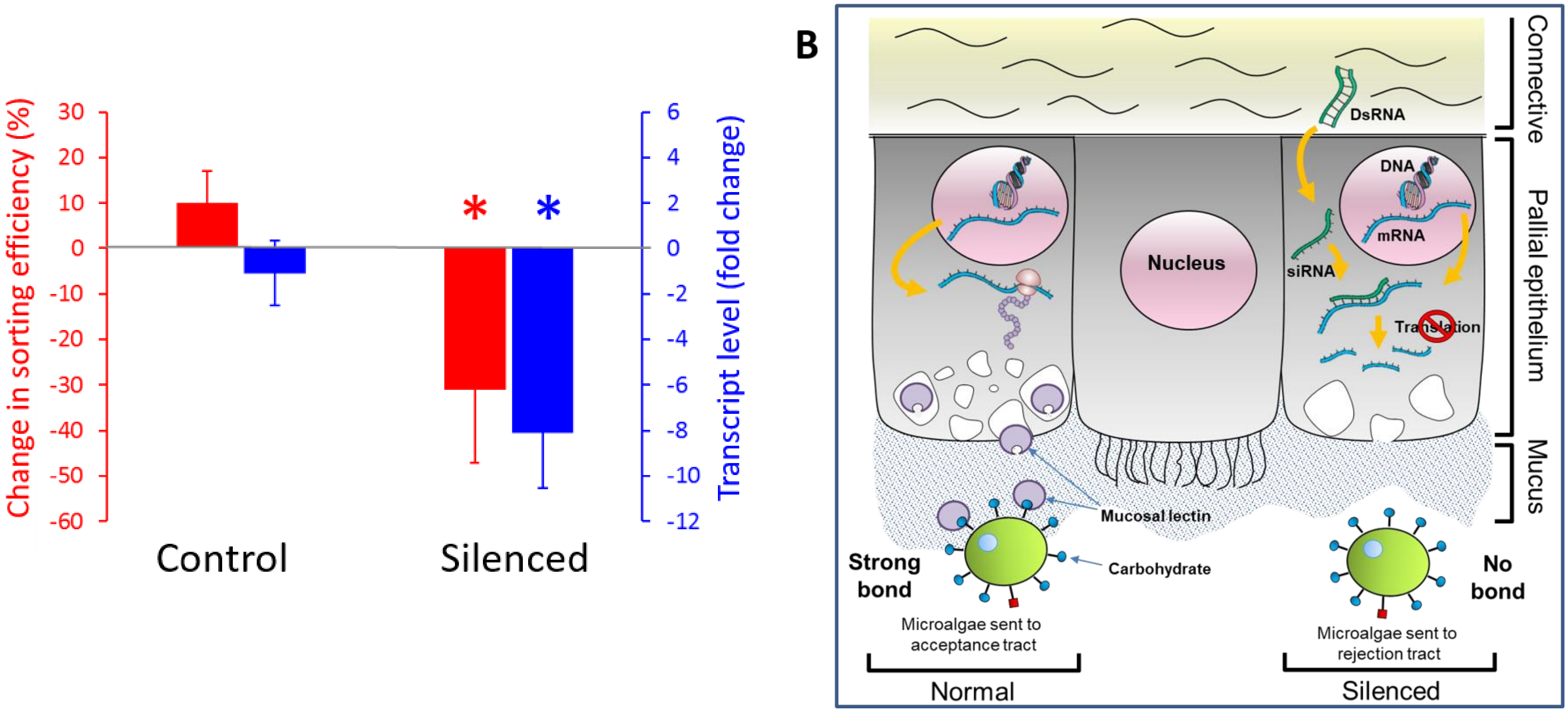
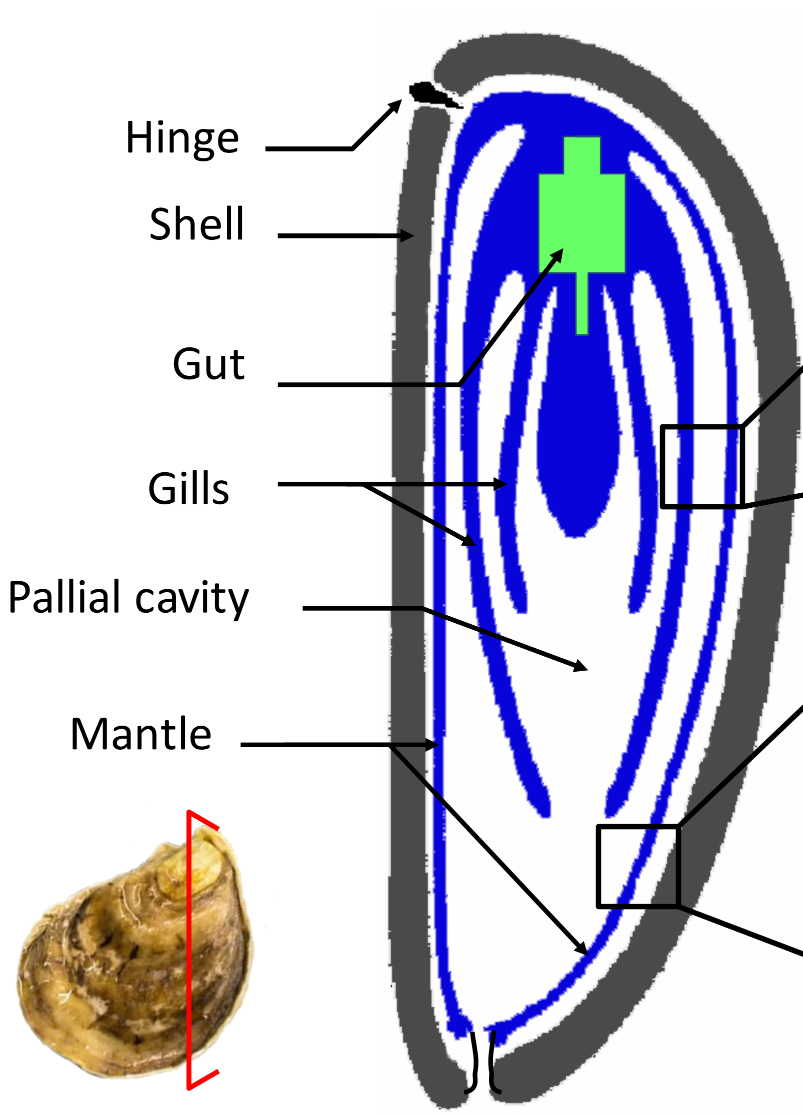
RESEARCH ARTICLE

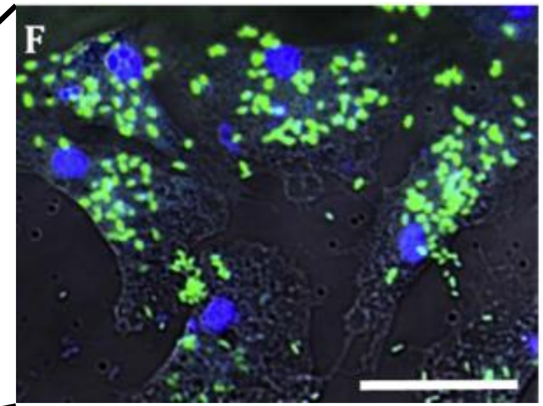
Reverse genetics demonstrate the role of mucosal C-type lectins in food particle selection in the oyster *Crassostrea virginica*

Emmanuelle Pales Espinosa* and Bassem Allam

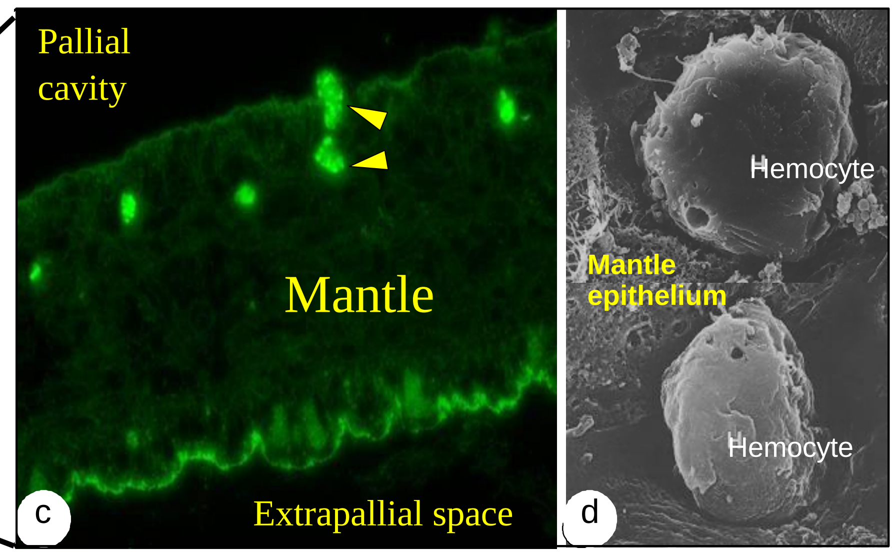


Mucus covering pallial surfaces also contains blood cells (hemocytes)





Lau et al. 2018a and b



Fluorescence picture by Andy Mount

Allam, 2008

Fish and Shellfish Immunology
journal homepage: www.elsevier.com/locate/fsi

Full length article

Characterization of hemocytes from different body fluids of the eastern oyster *Crassostrea virginica*

Yuk-Ting Lau, Lauren Sussman, Emmanuelle Pales Espinosa, Selma Katalay[1], Bassem Allam*

Journal of Invertebrate Pathology
journal homepage: www.elsevier.com/locate/jip

Transepithelial migration of mucosal hemocytes in *Crassostrea virginica* and potential role in *Perkinsus marinus* pathogenesis

Yuk-Ting Lau, Laura Gambino, Bianca Santos, Emmanuelle Pales Espinosa, Bassem Allam*

Fish and Shellfish Immunology
journal homepage: www.elsevier.com/locate/fsi

Full length article

Regulation of oyster (*Crassostrea virginica*) hemocyte motility by the intracellular parasite *Perkinsus marinus*: A possible mechanism for host infection

Yuk-Ting Lau, Laura Gambino, Bianca Santos, Emmanuelle Pales Espinosa, Bassem Allam*

Fish and Shellfish Immunology
journal homepage: www.elsevier.com/locate/fsi

Full length article

Regulation of apoptosis-related genes during interactions between oyster hemocytes and the alveolate parasite *Perkinsus marinus*

Yuk-Ting Lau, Bianca Santos, Michelle Barbosa, Emmanuelle Pales Espinosa, Bassem Allam*

Conclusions

- Mucosal interfaces contain a wide range of immune factors
- Specific host-microbe interactions at these interfaces are determinant for some of the most basic physiological processes in bivalves
- Some of these non-self recognition factors have been diverted for the optimization of energy gains
- Hemocytes associated with mucosal surfaces occupy a niche similar to that occupied by dendritic cells in vertebrates

Acknowledgements

- Co-PI and co-author: Emmanuelle Pales Espinosa

- Funding: National Science Foundation

- Many students, in particular Joyce Lau, Wade Carden, Sarah Winnicki, Michelle Barbosa and Anna Hollembeak

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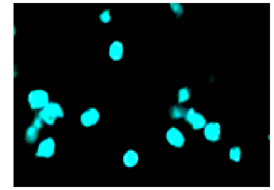
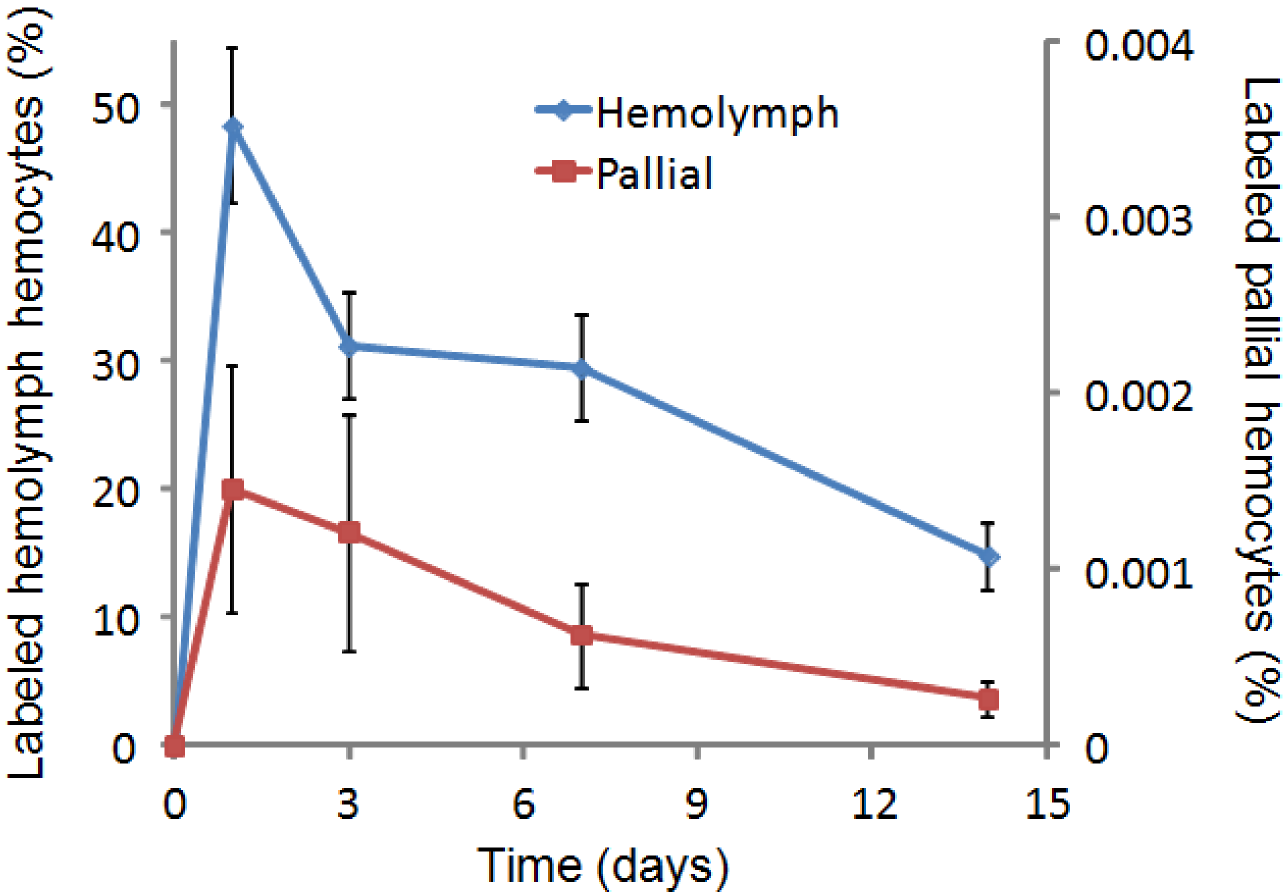
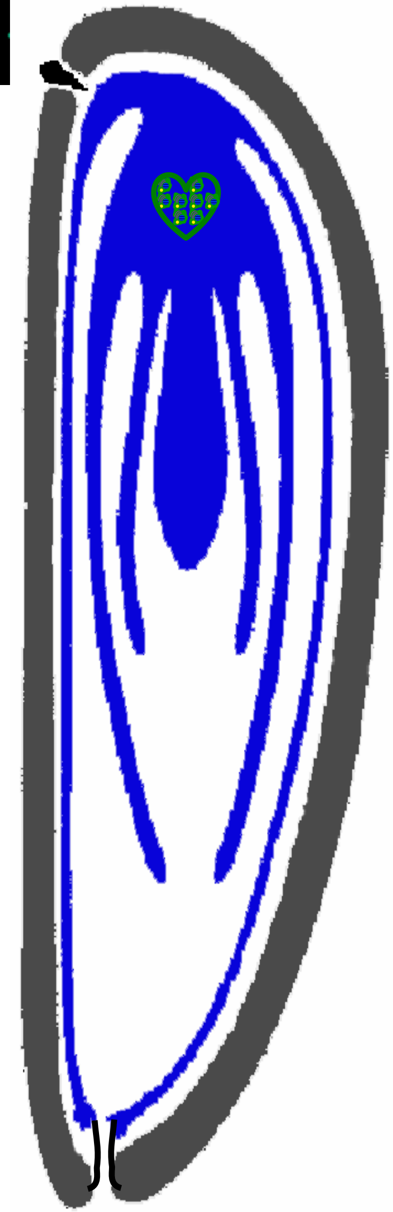
MADL - 2019

Exchange exists between circulatory and pallial hemocytes



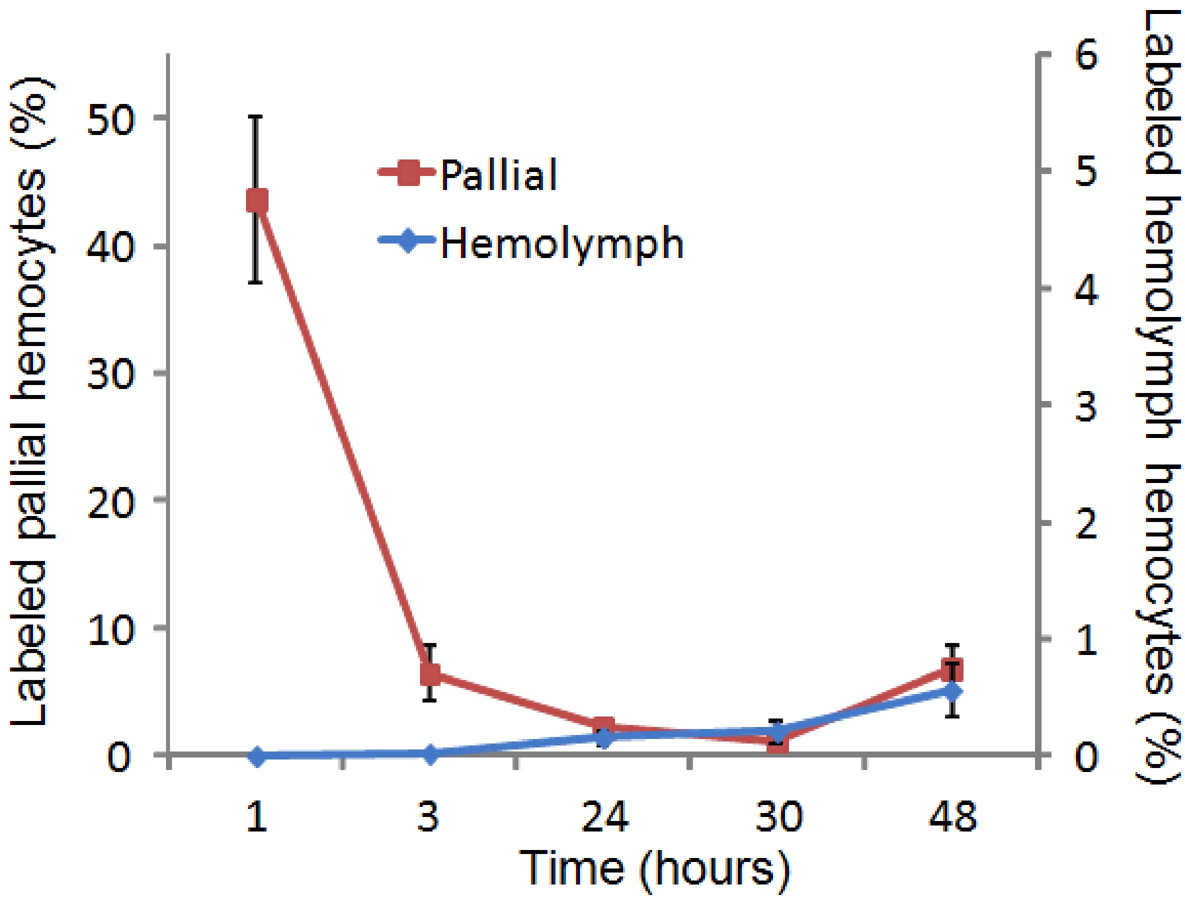


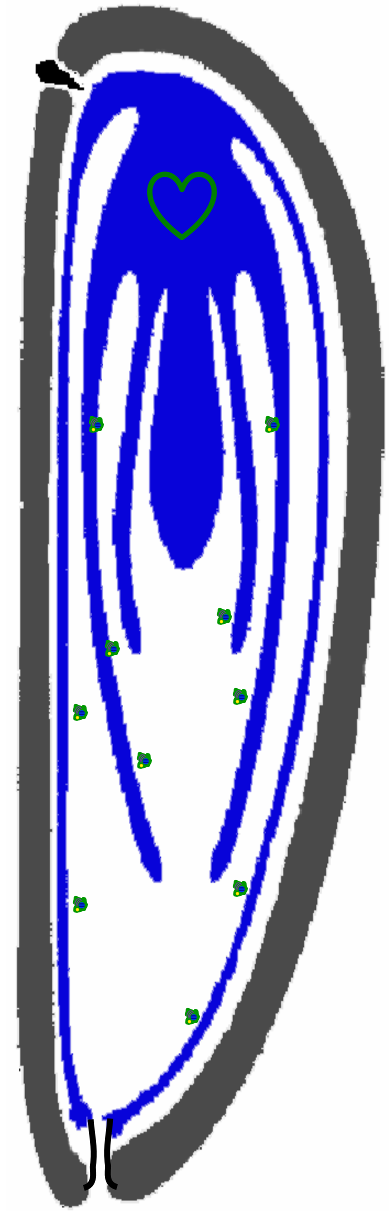


Tracking of hemolymph hemocytes. Fluorescent hemocytes peaked in both compartments at day 1. N=11 to 16 oysters/data point.

Lau et al., in preparation

Pallial hemocytes can also reach the circulatory system

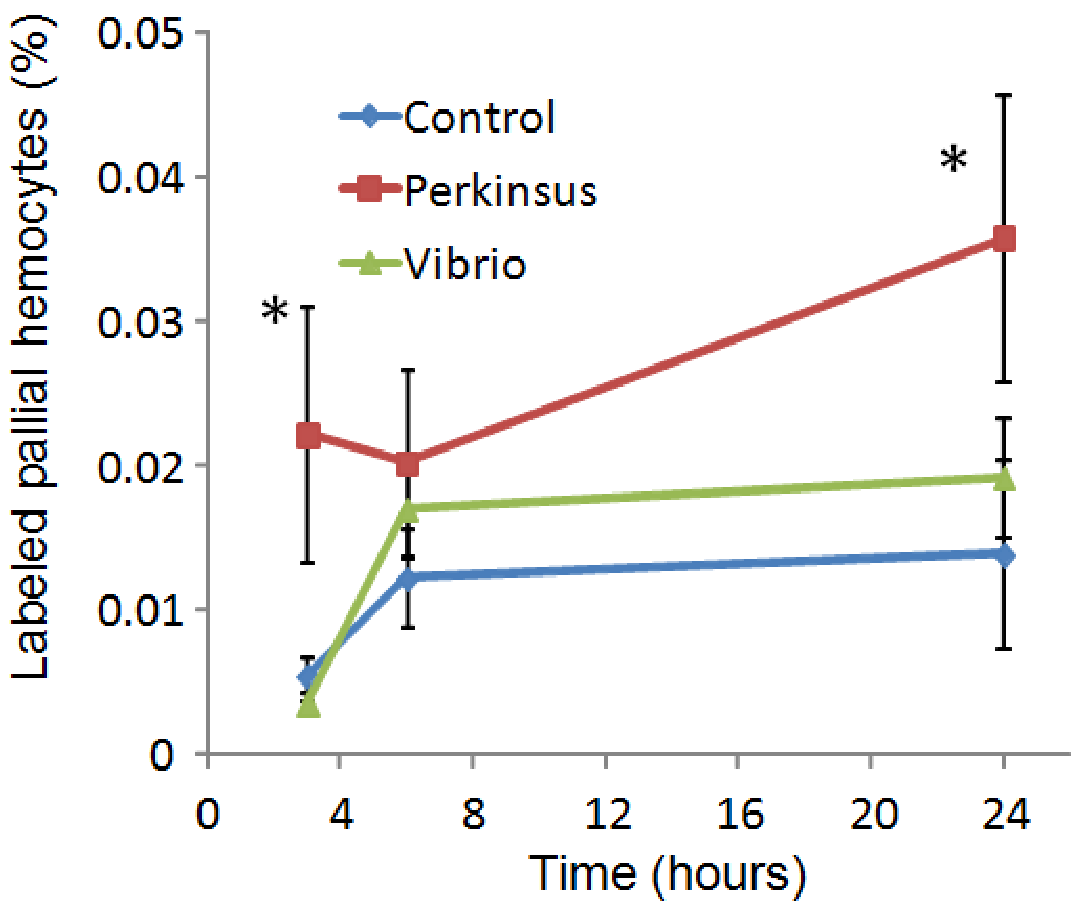


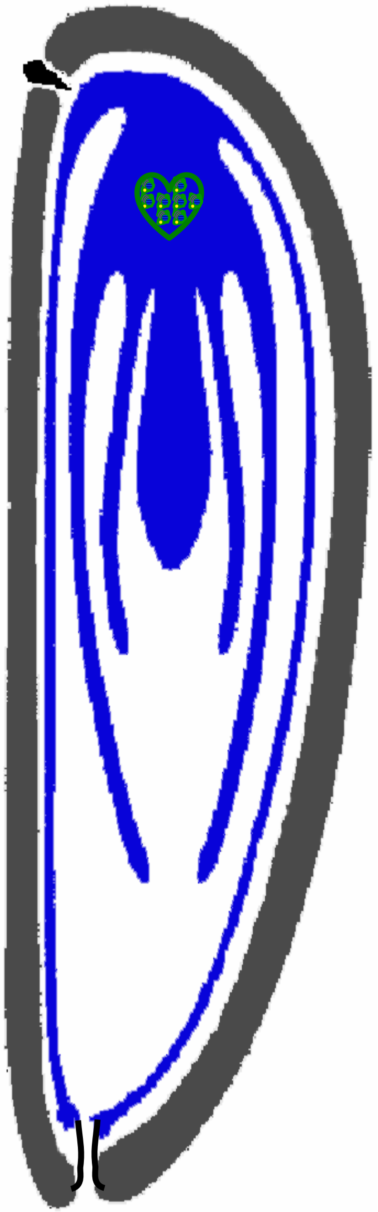


Tracking of pallial hemocytes. Fluorescent hemocytes were visible in the circulatory system on day 1 and peaked after 48 hours. N=10 oysters/ data point.

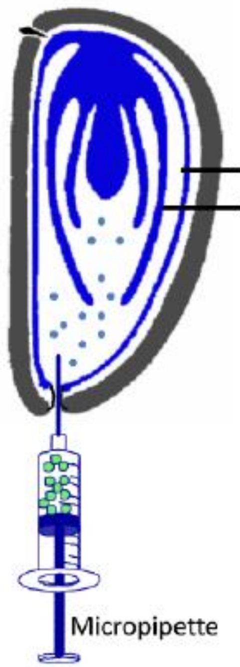
Lau et al., in preparation

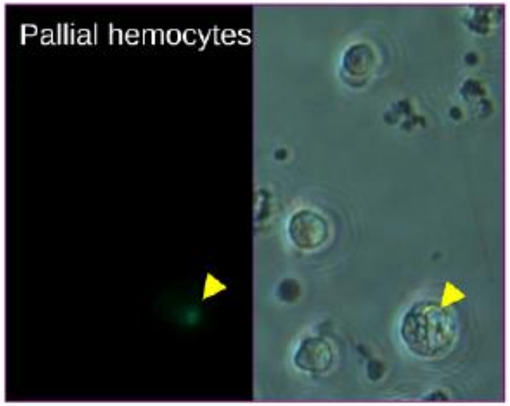
Oyster exposure to *Perkinsus marinus* increases hemocyte trafficking

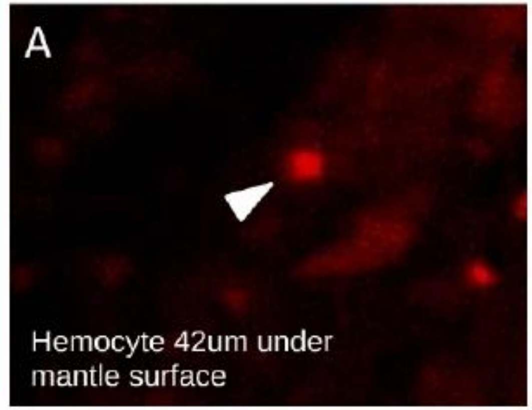


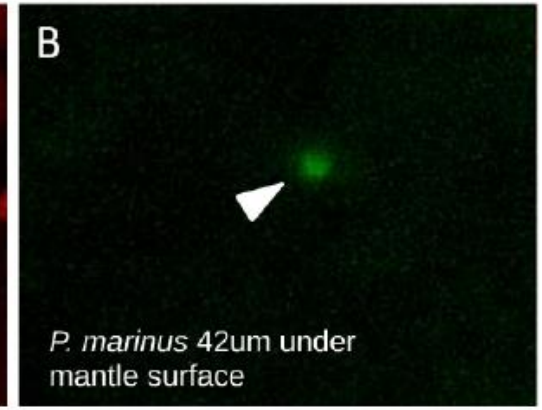


New question: can the parasite take advantage of hemocyte trafficking to get access into host tissues?









P. marinus cells can be internalized by pallial hemocytes and transferred to hemolymph