

The nephrocomplex (formerly known as ‘antennal gland’),
the golden gate for pathogens
& central engine for the molting process

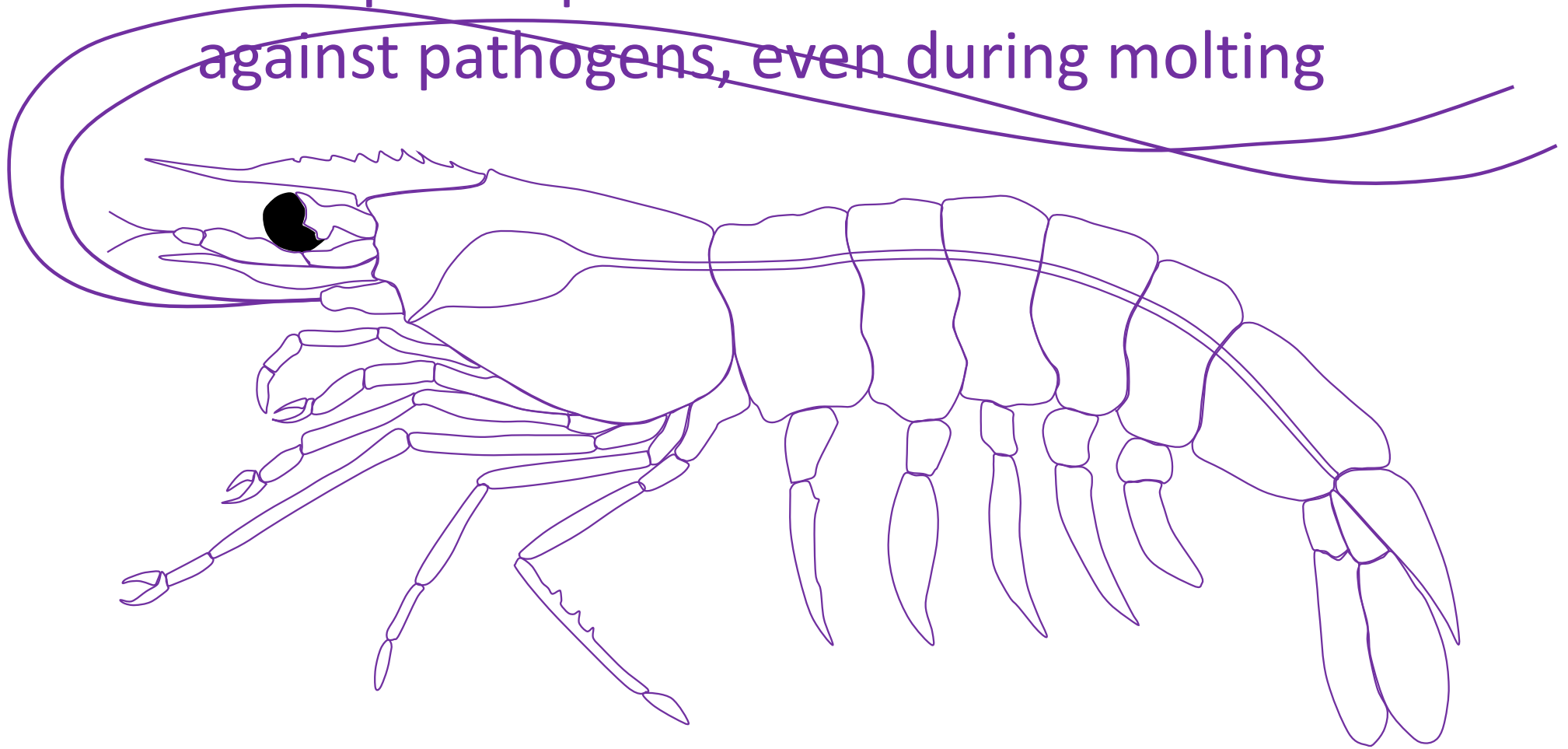
Hans Nauwynck

The shrimp nephrocomplex serves as a major portal of pathogen entry
and is involved in the molting process

Gaëtan De Gryse & Thuong Van Khuong, Benedicte Descamps, Wim Van Den Broeck, Christian Vanhove,
Pieter Cornillie, Patrick Sorgeloos, Peter Bossier and Hans Nauwynck

<https://doi.org/10.1073/pnas.2013518117>

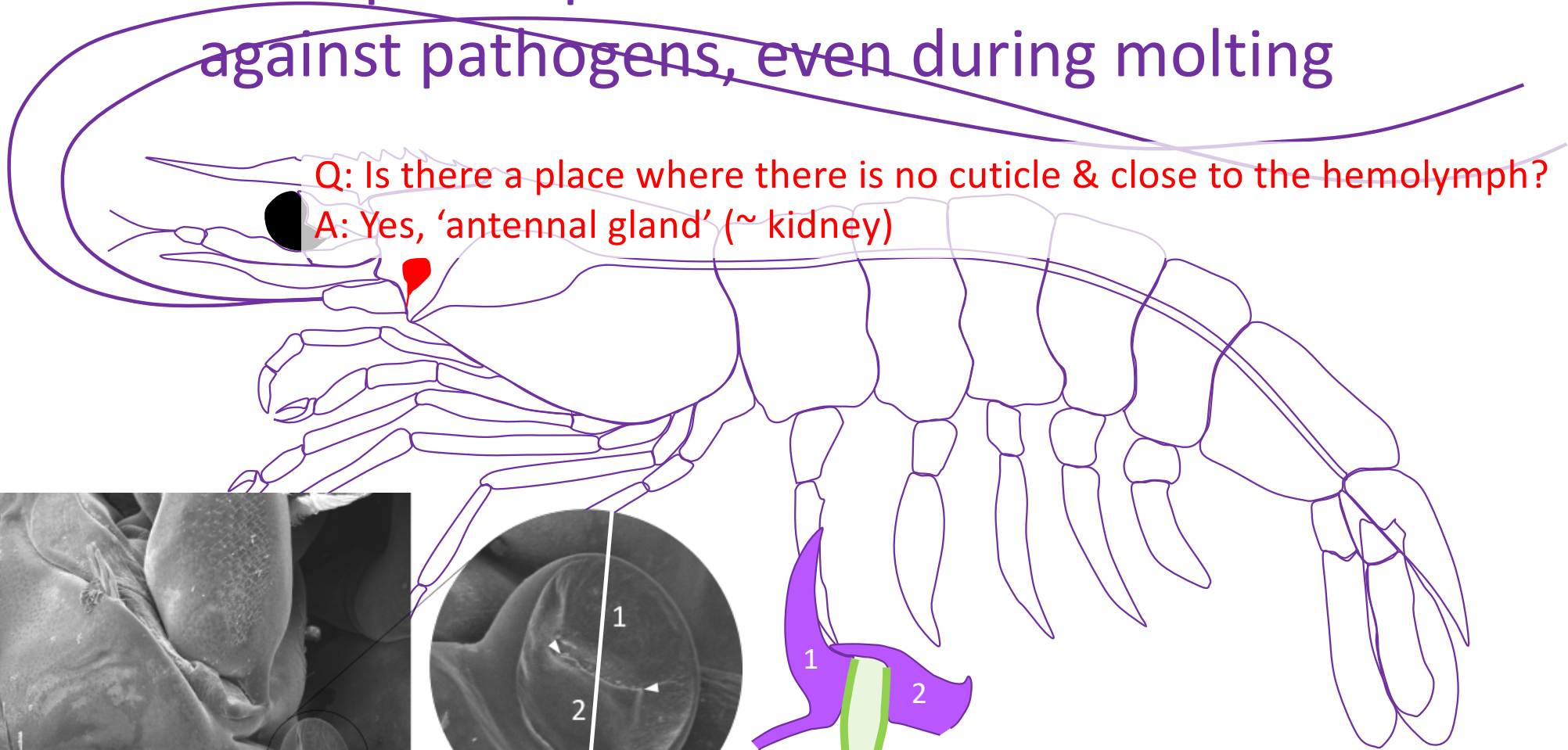
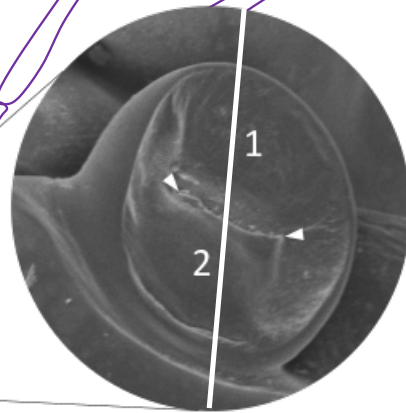
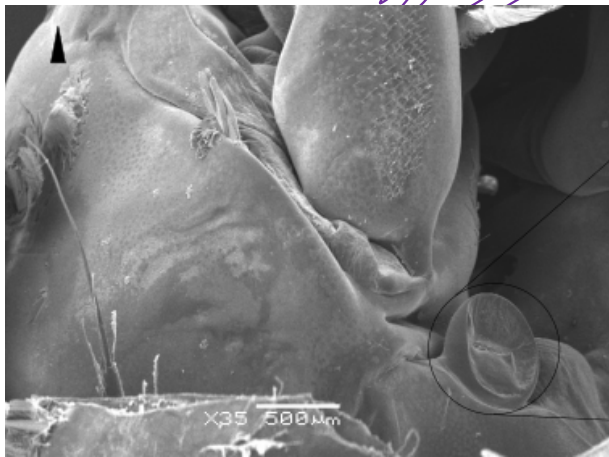
The cuticle & peritrophic membrane form a firm barrier against pathogens, even during molting



The cuticle & peritrophic membrane form a firm barrier against pathogens, even during molting

Q: Is there a place where there is no cuticle & close to the hemolymph?

A: Yes, 'antennal gland' (~ kidney)



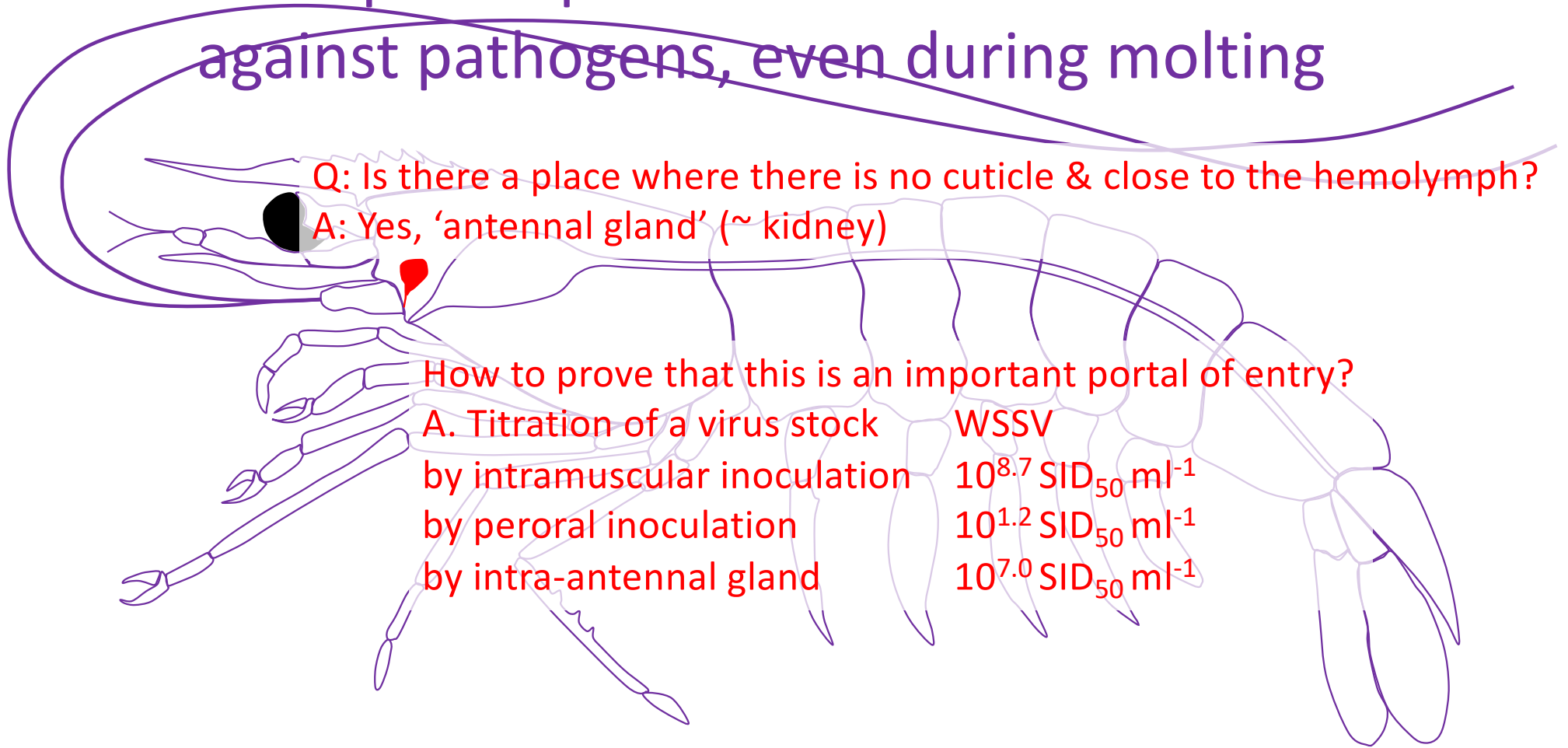
The cuticle & peritrophic membrane form a firm barrier against pathogens, even during molting

Q: Is there a place where there is no cuticle & close to the hemolymph?

A: Yes, 'antennal gland' (~ kidney)

How to prove that this is an important portal of entry?

A. Titration of a virus stock	WSSV
by intramuscular inoculation	$10^{8.7} \text{ SID}_{50} \text{ ml}^{-1}$
by peroral inoculation	$10^{1.2} \text{ SID}_{50} \text{ ml}^{-1}$
by intra-antennal gland	$10^{7.0} \text{ SID}_{50} \text{ ml}^{-1}$



The cuticle & peritrophic membrane form a firm barrier against pathogens, even during molting

Q: Is there a place where there is no cuticle & close to the hemolymph?

A: Yes, 'antennal gland' (~ kidney)

How to prove that this is an important portal of entry?

A. Titration of a virus stock	WSSV
by intramuscular inoculation	$10^{8.7} \text{ SID}_{50} \text{ ml}^{-1}$
by peroral inoculation	$10^{1.2} \text{ SID}_{50} \text{ ml}^{-1}$
by intra-antennal gland	$10^{7.0} \text{ SID}_{50} \text{ ml}^{-1}$

Compared to the intramuscular route,

- only 56 times more virus is needed to infect shrimp via the intra-antennal gland inoculation
- 28,800,000 more virus is needed to infect shrimp via peroral inoculation

The cuticle & peritrophic membrane form a firm barrier against pathogens, even during molting

Q: Is there a place where there is no cuticle & close to the hemolymph?

A: Yes, 'antennal gland' (~ kidney)

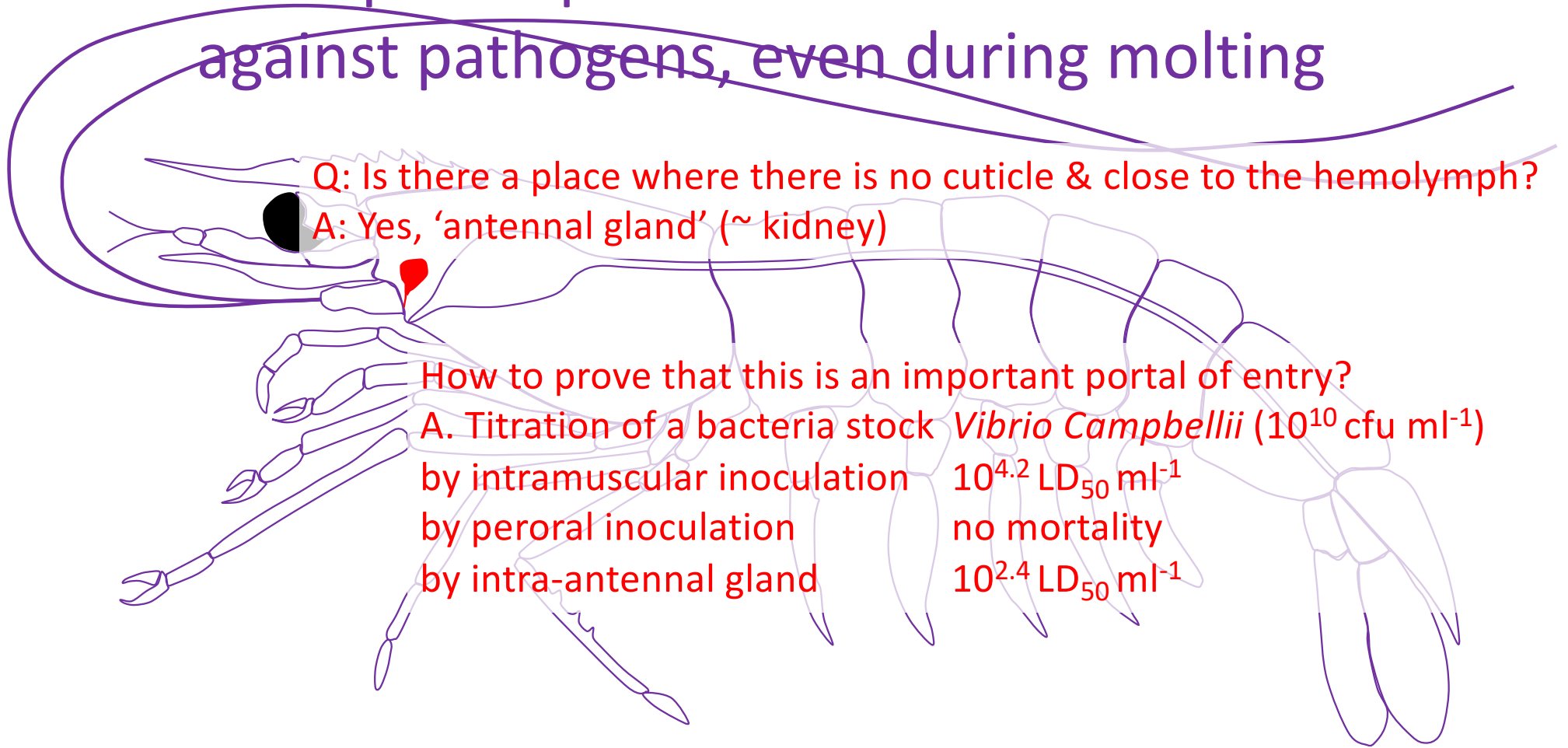
How to prove that this is an important portal of entry?

A. Titration of a bacteria stock *Vibrio Campbellii* (10^{10} cfu ml⁻¹)

by intramuscular inoculation $10^{4.2}$ LD₅₀ ml⁻¹

by peroral inoculation no mortality

by intra-antennal gland $10^{2.4}$ LD₅₀ ml⁻¹



The cuticle & peritrophic membrane form a firm barrier against pathogens, even during molting

Q: Is there a place where there is no cuticle & close to the hemolymph?

A: Yes, 'antennal gland' (~ kidney)

How to prove that this is an important portal of entry?

A. Titration of a bacteria stock <i>Vibrio Campbellii</i> (10^{10} cfu ml ⁻¹)	
by intramuscular inoculation	$10^{4.2}$ LD ₅₀ ml ⁻¹
by peroral inoculation	no mortality
by intra-antennal gland	$10^{2.4}$ LD ₅₀ ml ⁻¹

Compared to the intramuscular route,

- only 62 times more *V. Campbellii* is required to kill shrimp via the intra-antennal gland inoculation
- no mortality with 10^9 *V. Campbellii* via peroral route

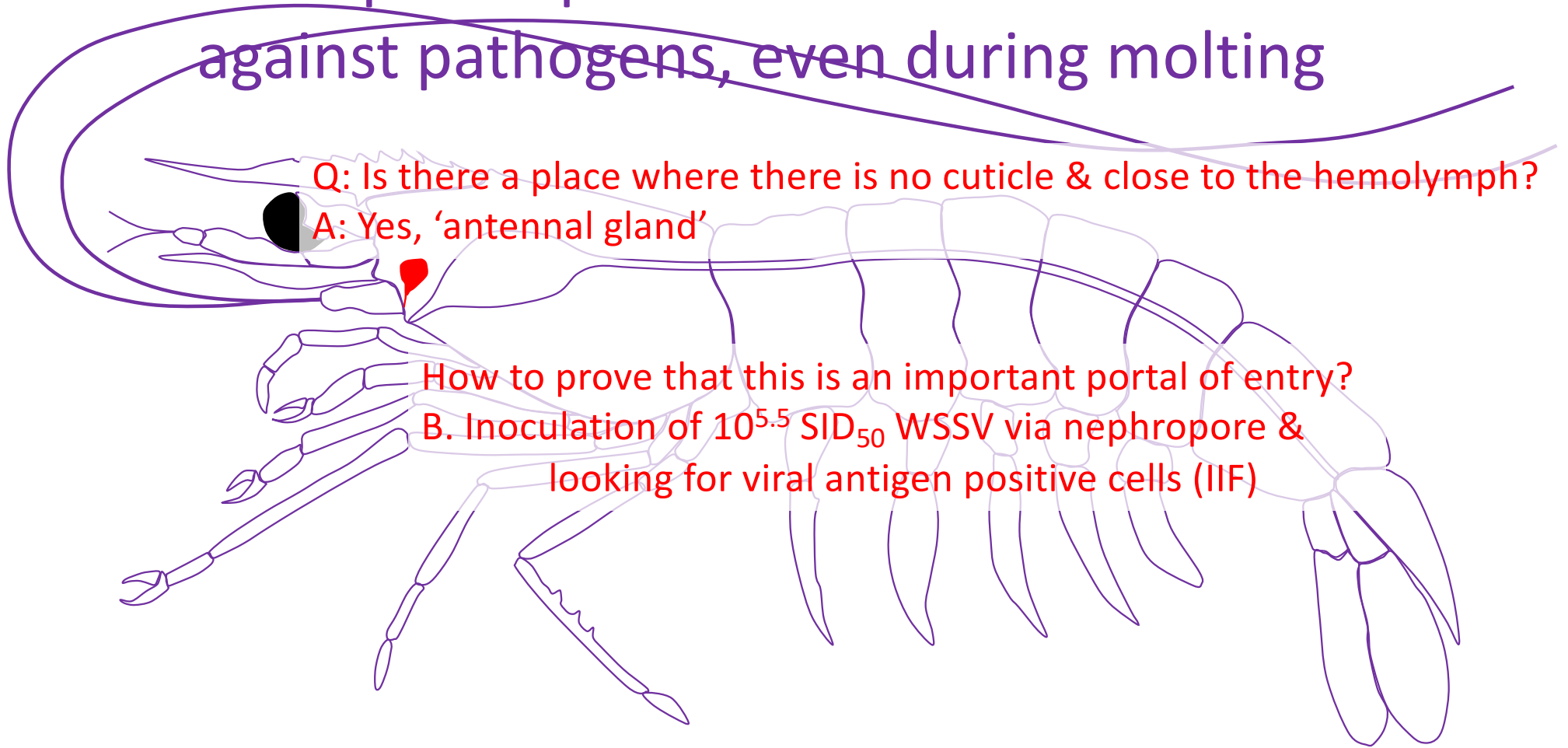
The cuticle & peritrophic membrane form a firm barrier against pathogens, even during molting

Q: Is there a place where there is no cuticle & close to the hemolymph?

A: Yes, 'antennal gland'

How to prove that this is an important portal of entry?

B. Inoculation of $10^{5.5}$ SID₅₀ WSSV via nephropore & looking for viral antigen positive cells (IIF)



The cuticle & peritrophic membrane form a firm barrier against pathogens, even during molting

Q: Is there a place where there is no cuticle & close to the hemolymph?

A: Yes, 'antennal gland'

How to prove that this is an important portal of entry?

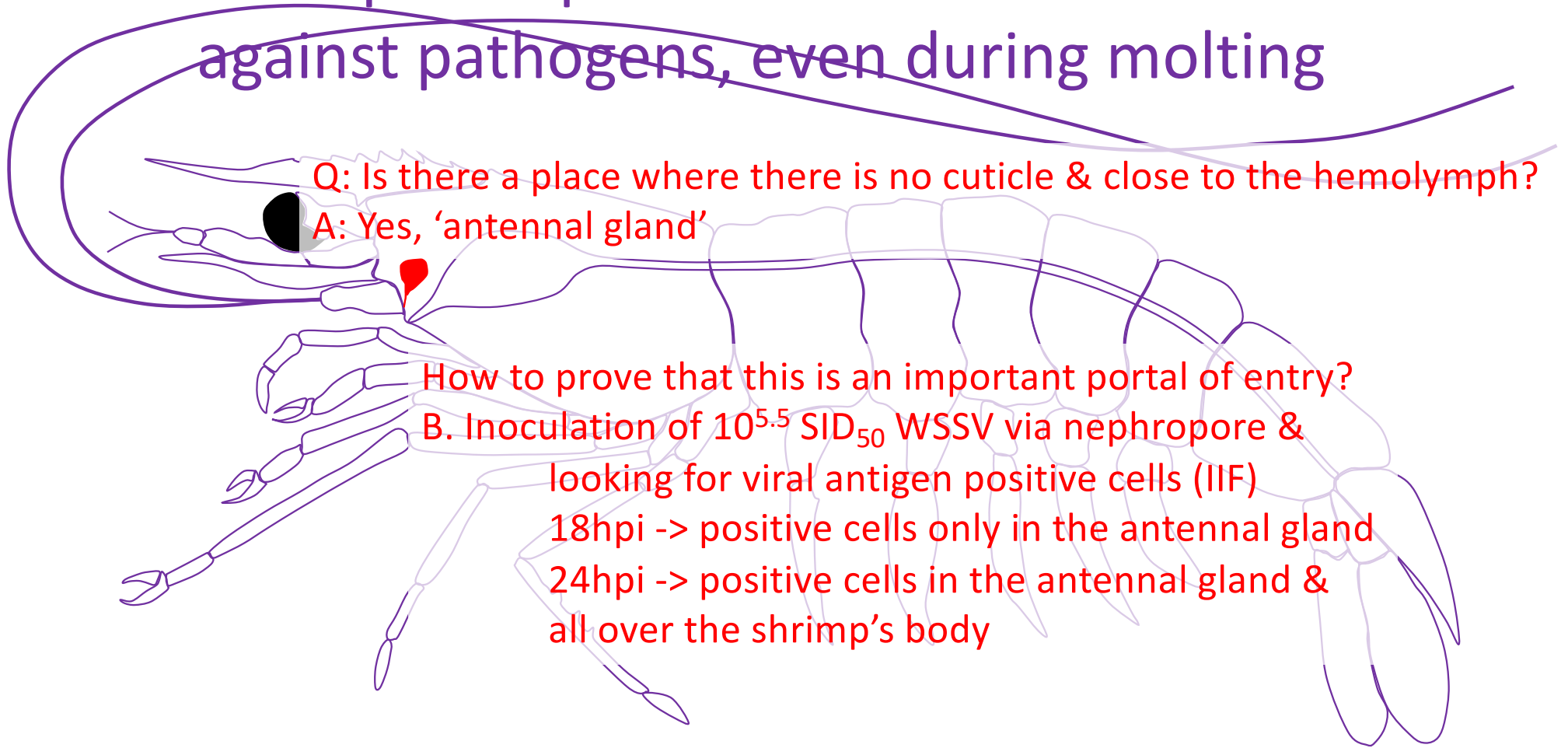
B. Inoculation of $10^{5.5}$ SID₅₀ WSSV via nephropore &

looking for viral antigen positive cells (IIF)

18hpi -> positive cells only in the antennal gland

24hpi -> positive cells in the antennal gland &

all over the shrimp's body



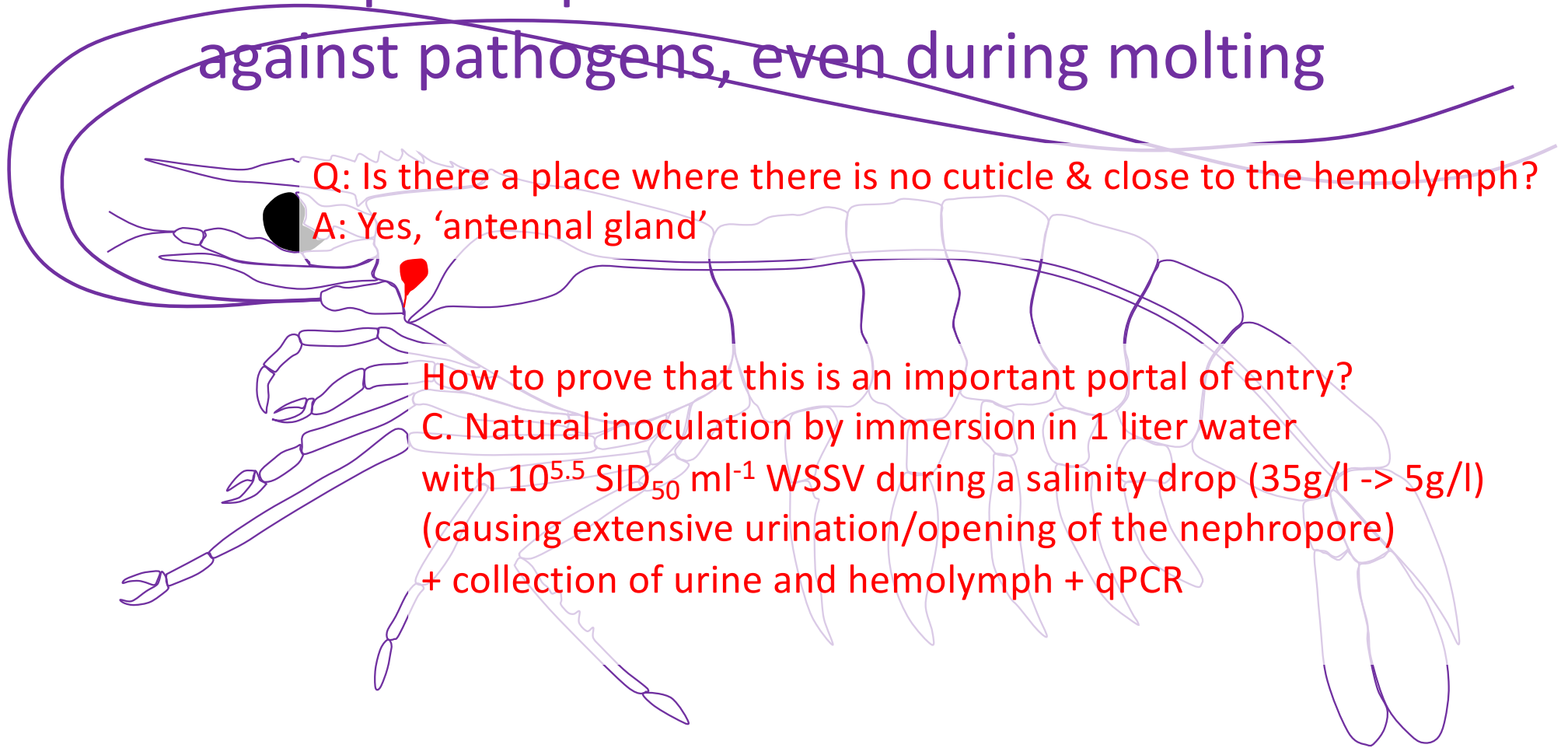
The cuticle & peritrophic membrane form a firm barrier against pathogens, even during molting

Q: Is there a place where there is no cuticle & close to the hemolymph?

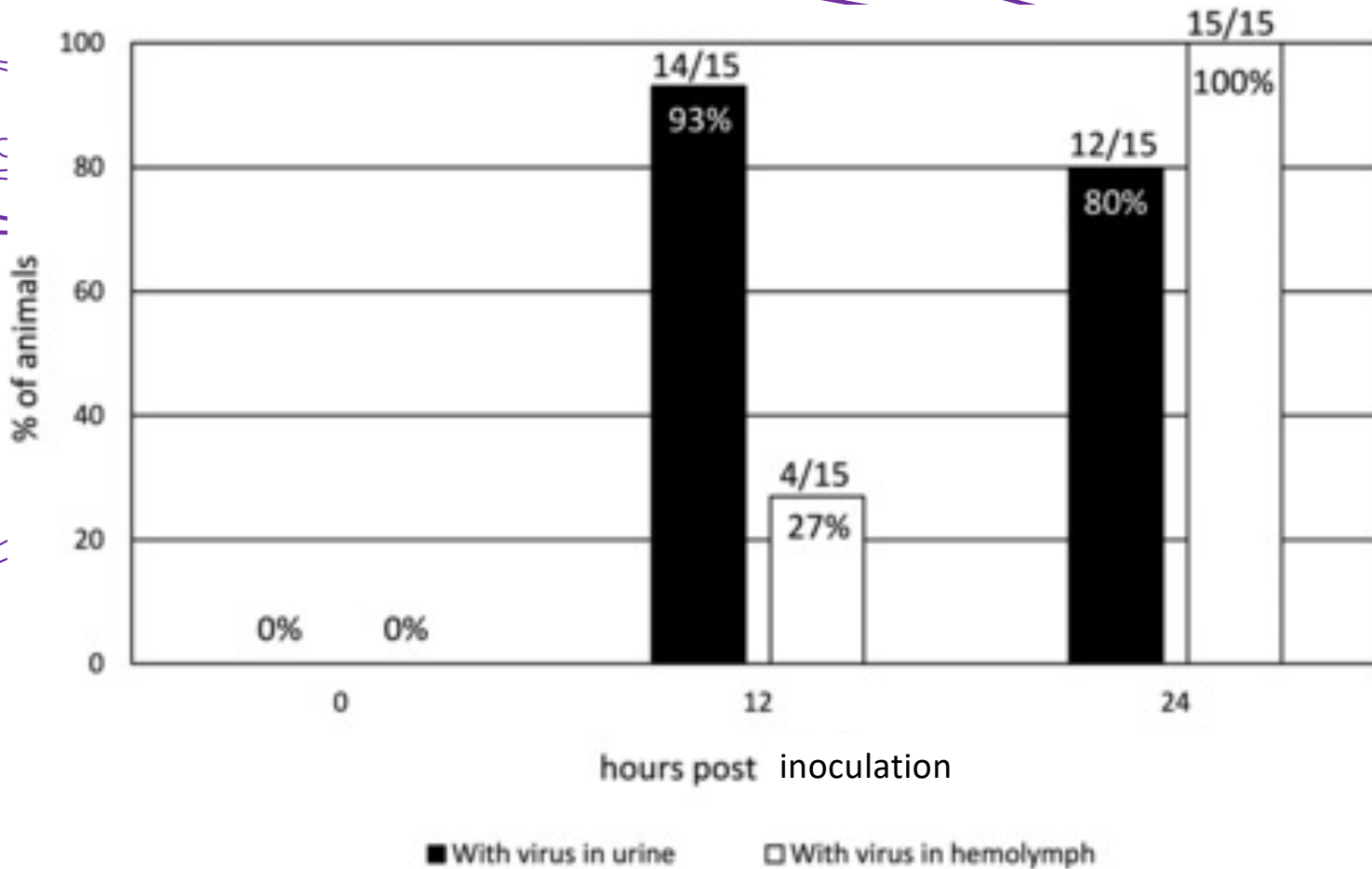
A: Yes, 'antennal gland'

How to prove that this is an important portal of entry?

C. Natural inoculation by immersion in 1 liter water with $10^{5.5}$ SID₅₀ ml⁻¹ WSSV during a salinity drop (35g/l -> 5g/l) (causing extensive urination/opening of the nephropore)
+ collection of urine and hemolymph + qPCR



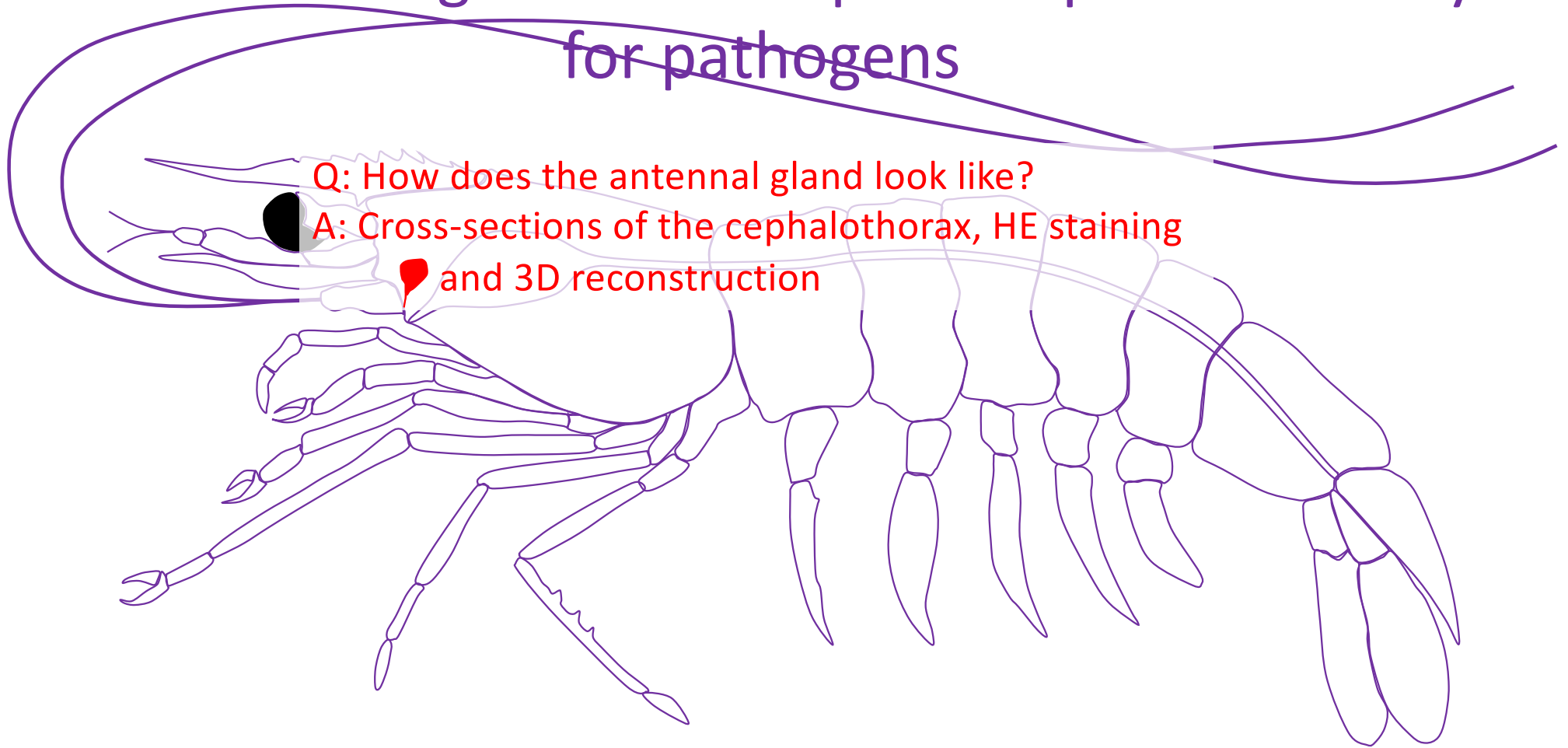
The cuticle & peritrophic membrane form a firm barrier against pathogens, even during molting



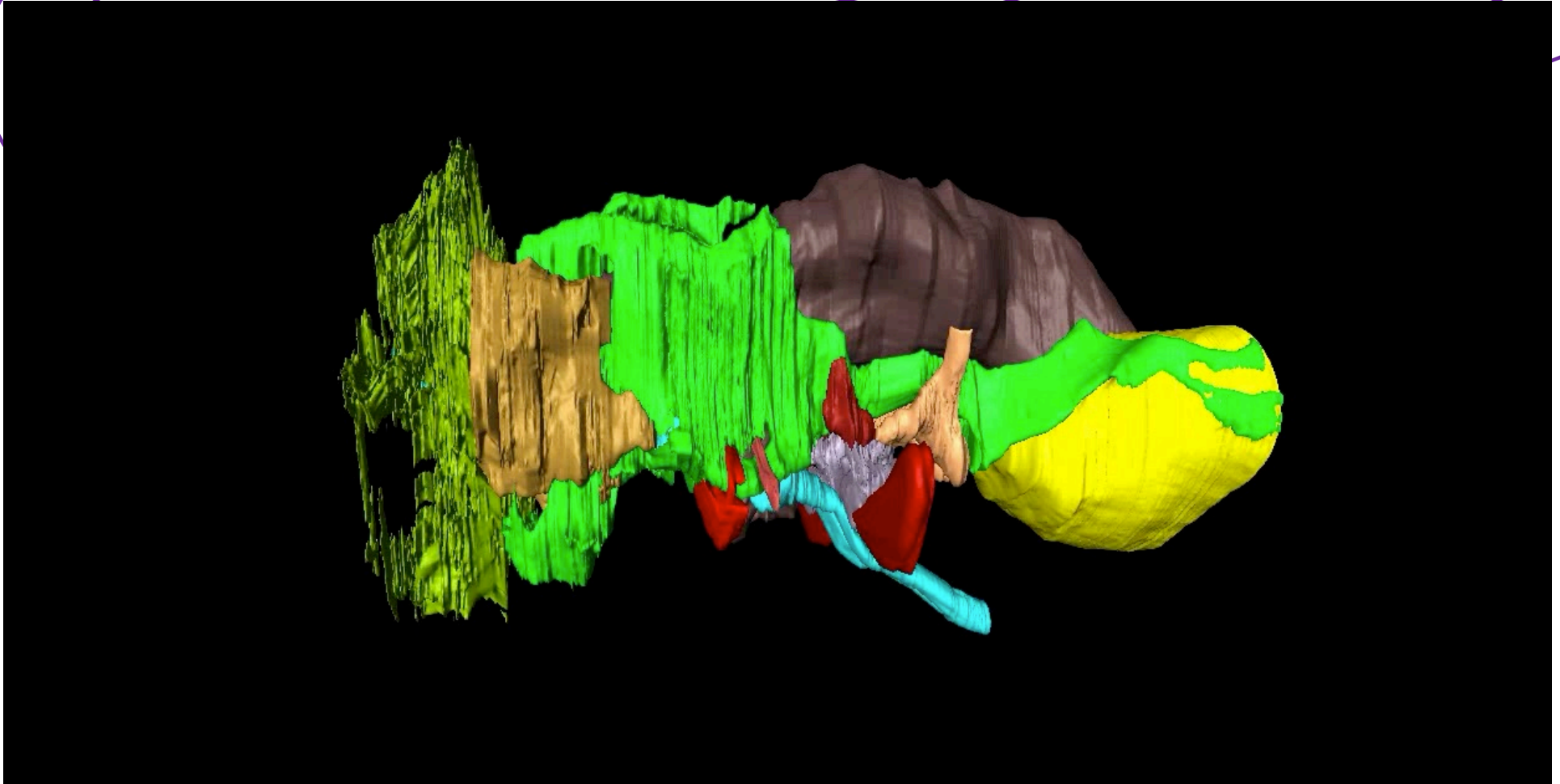
The antennal gland is an important portal of entry for pathogens

Q: How does the antennal gland look like?

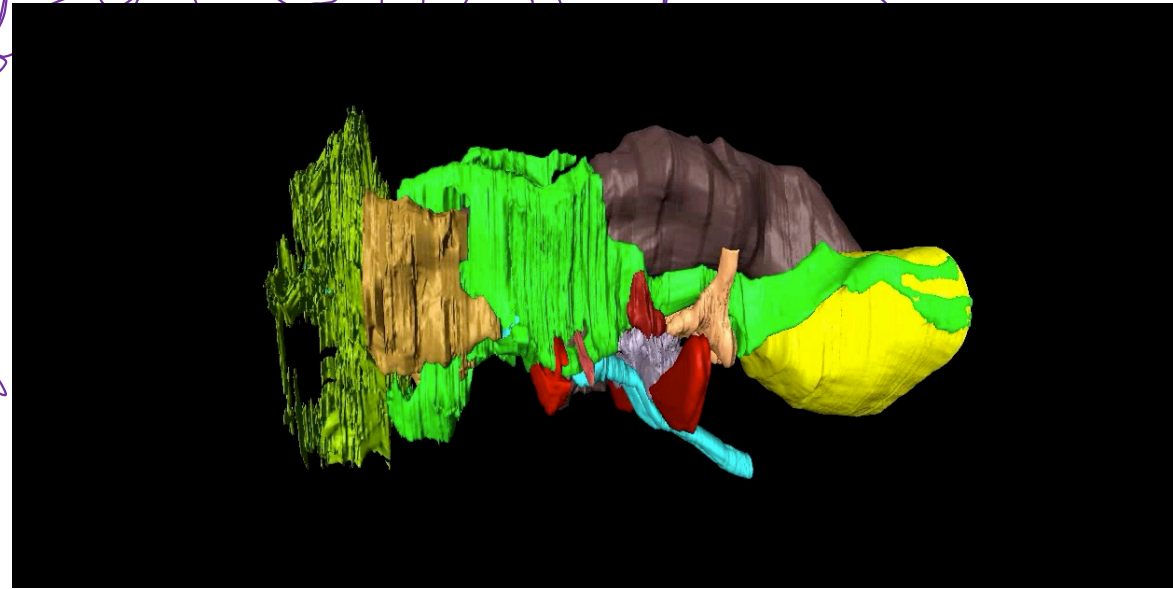
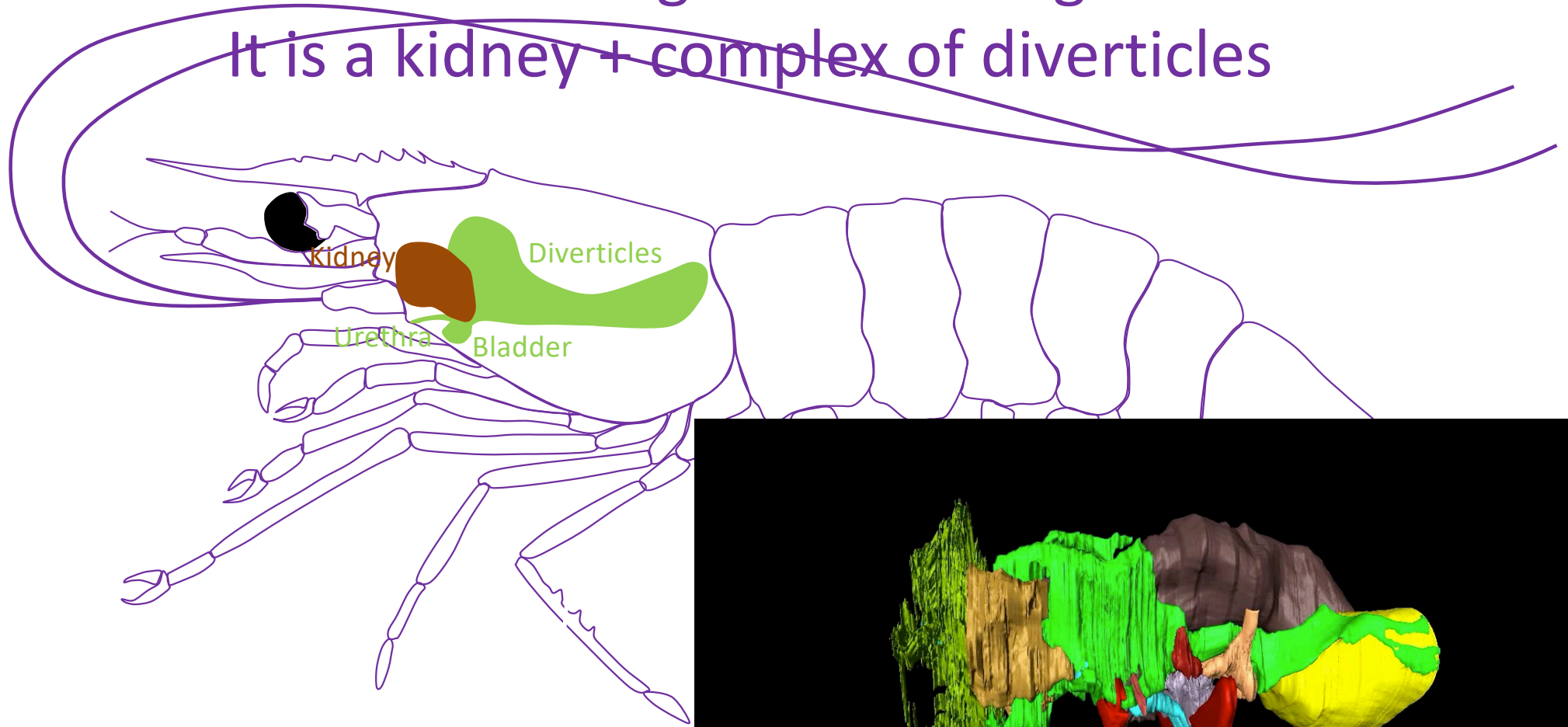
A: Cross-sections of the cephalothorax, HE staining
and 3D reconstruction



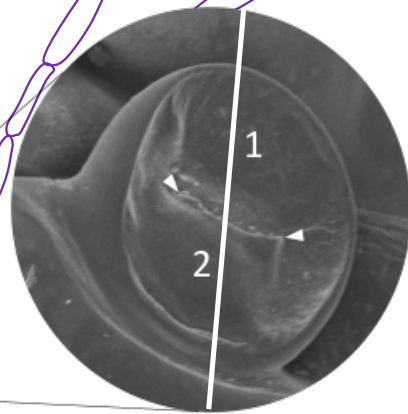
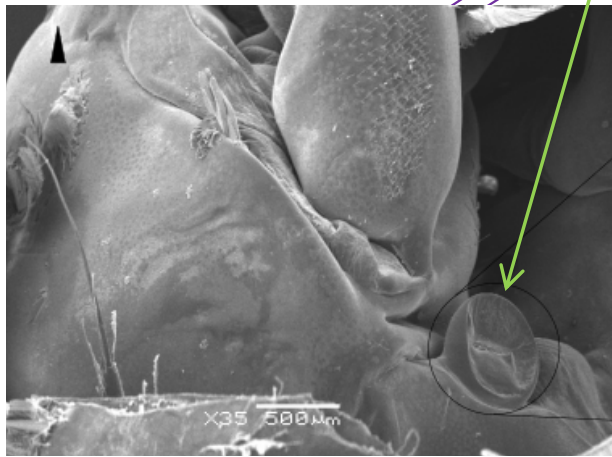
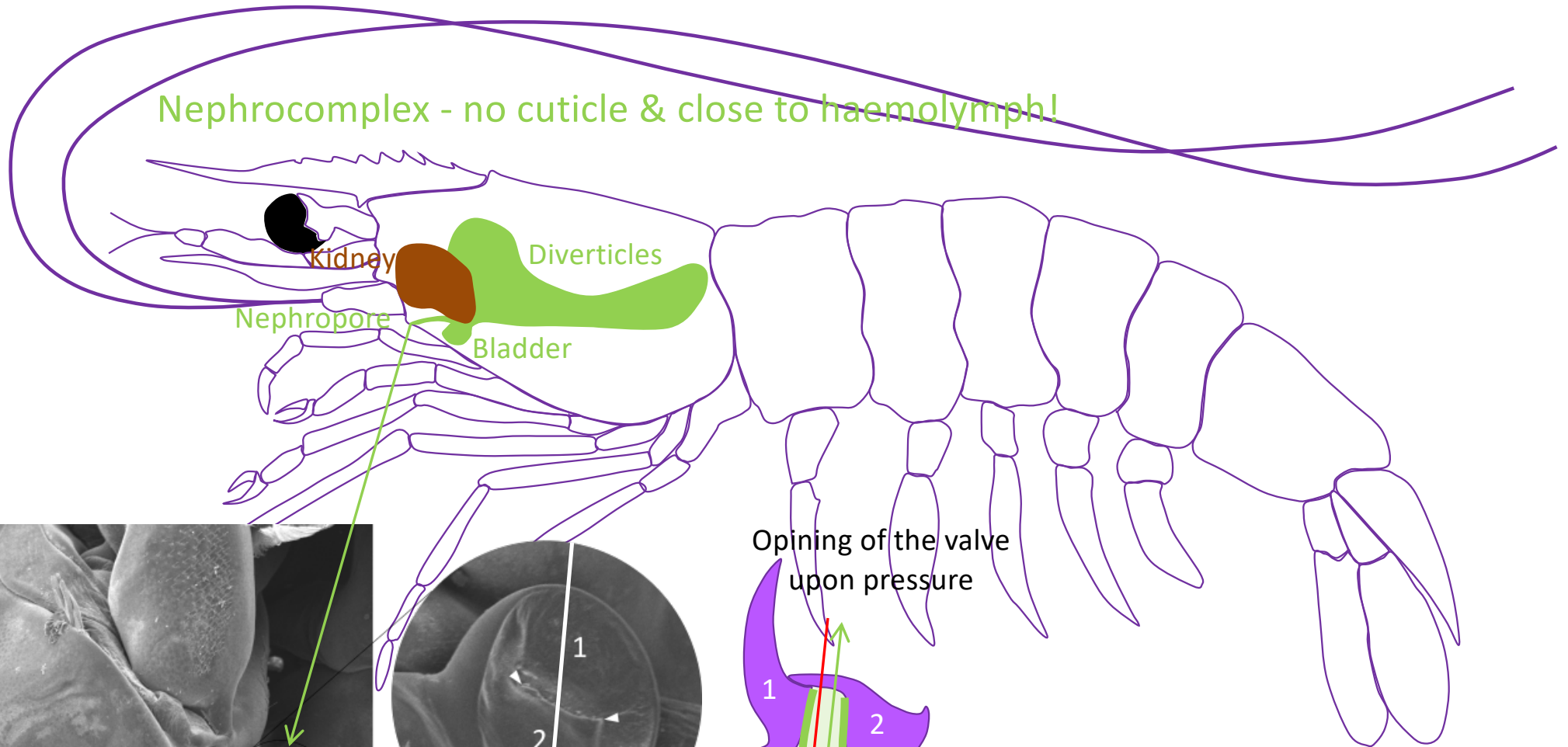
The antennal gland is an important portal of entry
for pathogens



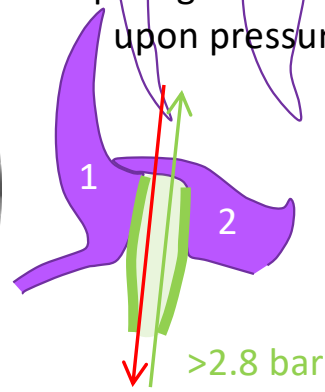
The antennal gland is not a gland!
It is a kidney + complex of diverticles



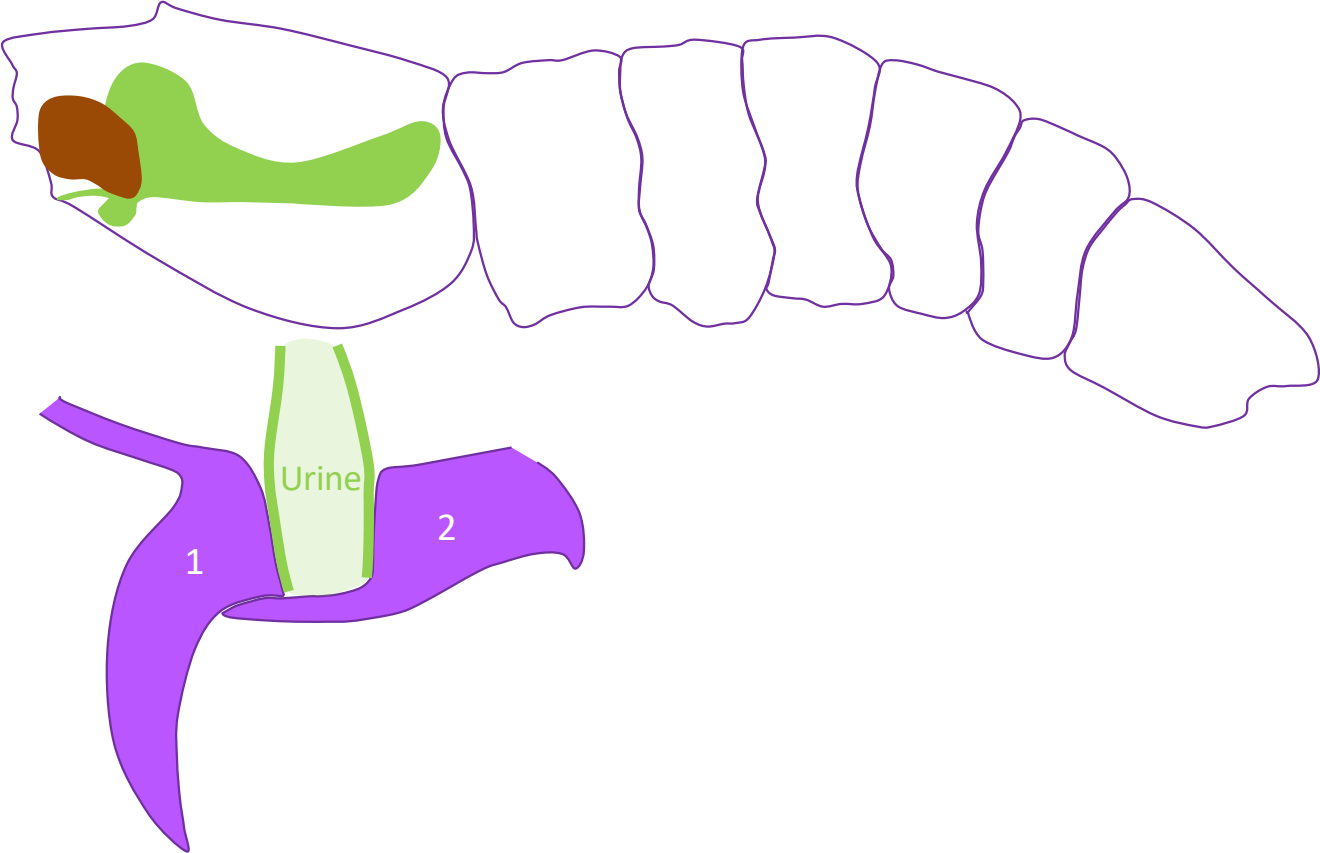
Nephrocomplex - no cuticle & close to haemolymph!



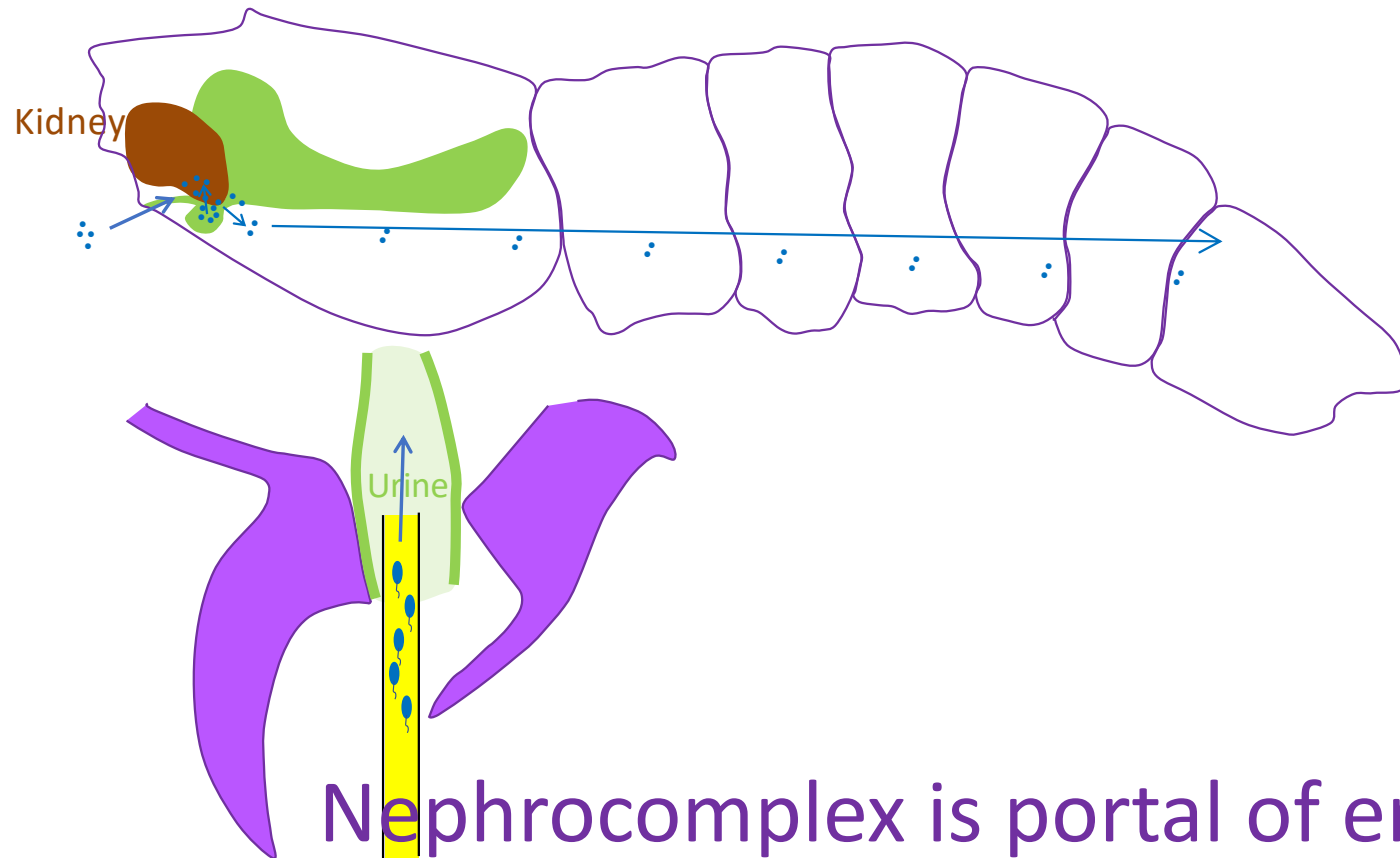
Opining of the valve upon pressure



MODEL 1: Portal of entry

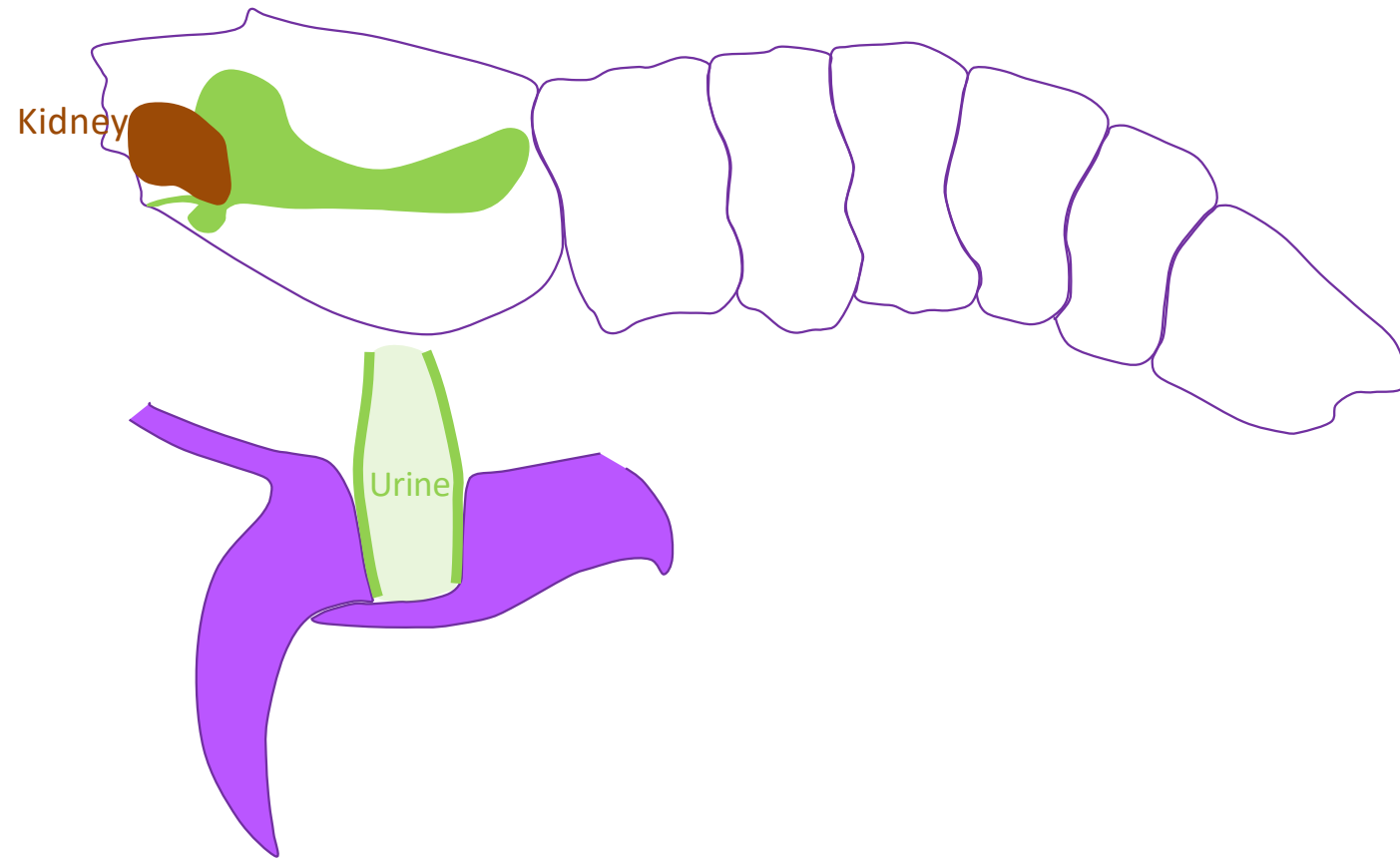


Intra-nephrocomplex inoculation of virus and bacteria -> infection!



Nephrocomplex is portal of entry!!!

How to enter under natural conditions?

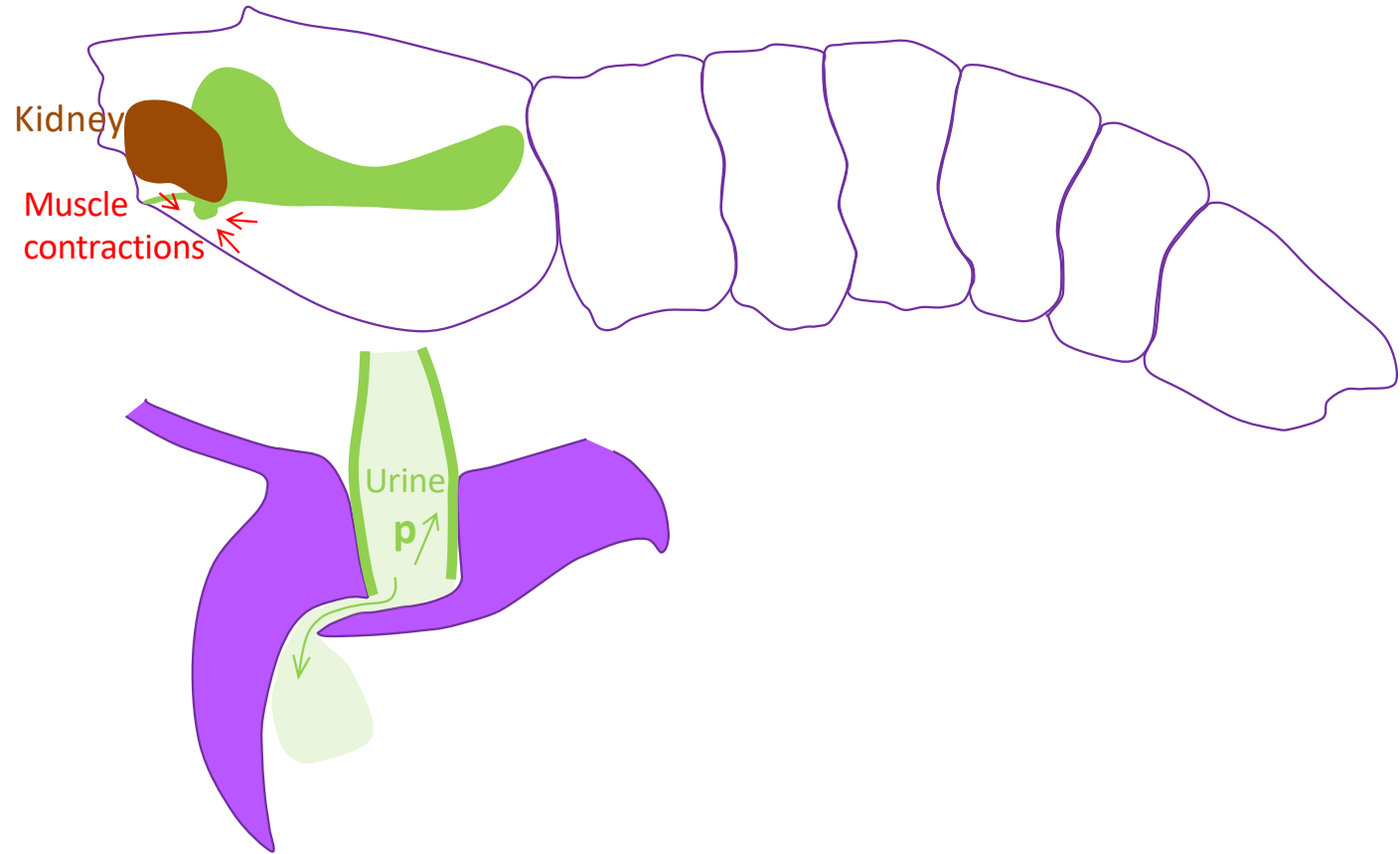


Urination!

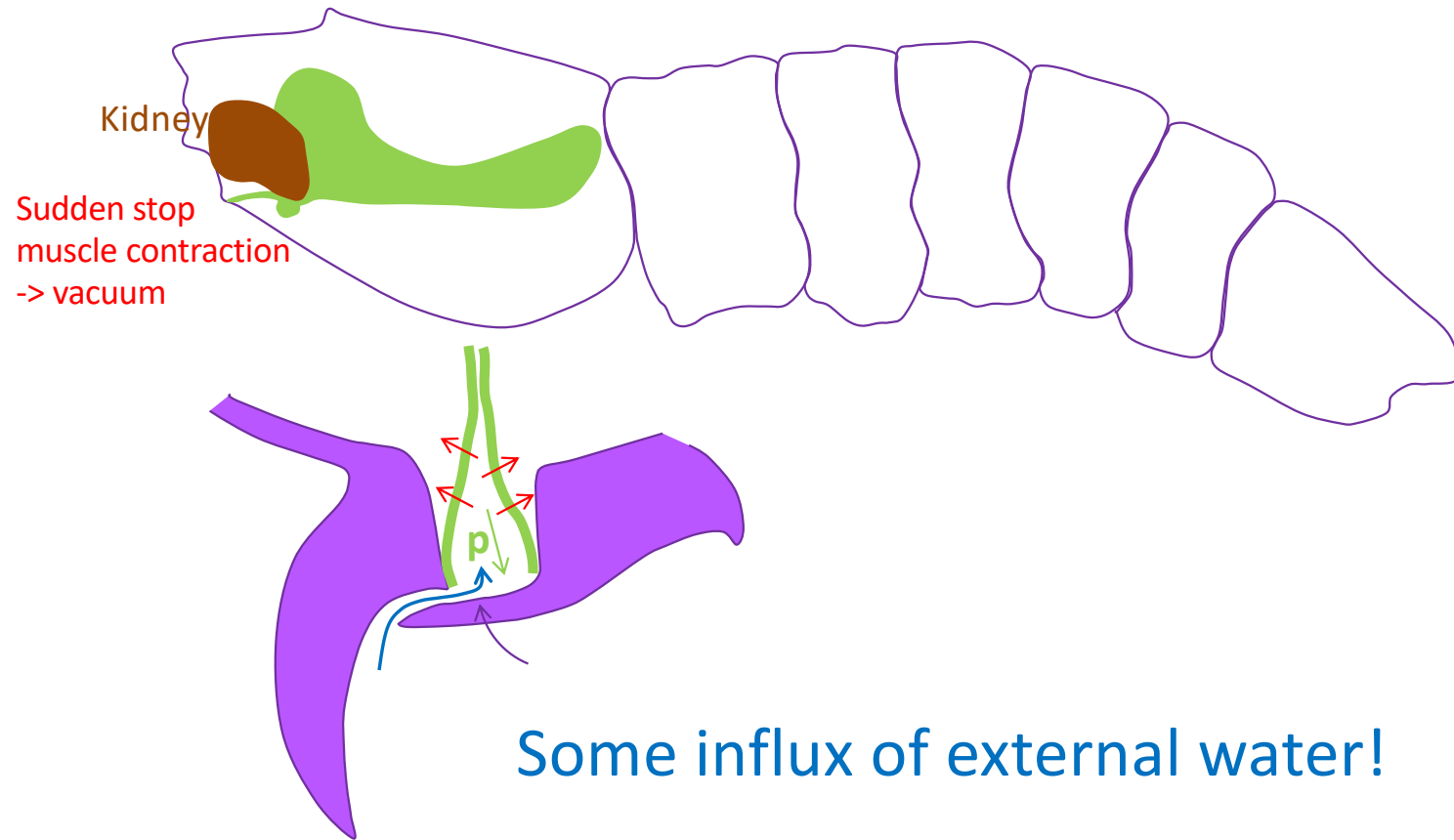
(hypotonic water, aggression, feed uptake, molting)

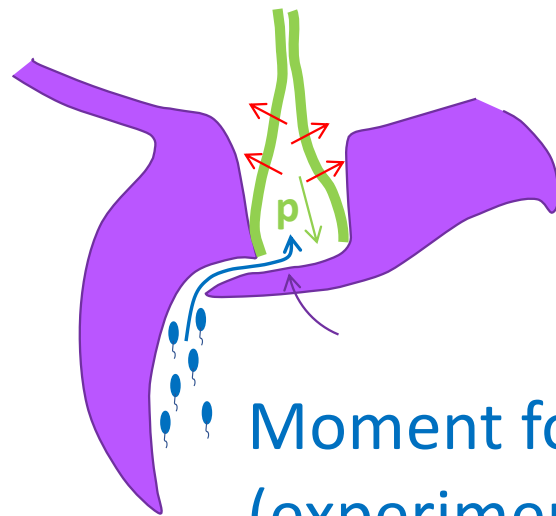
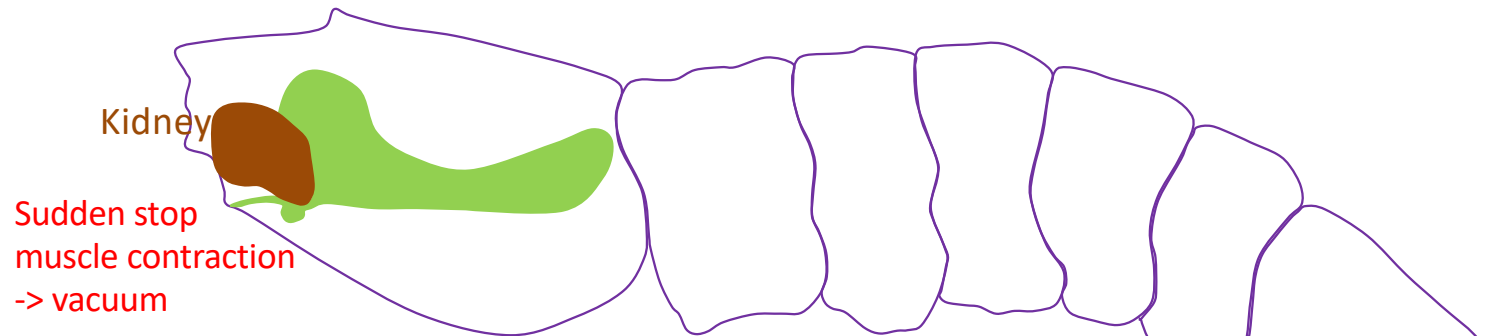


Urination

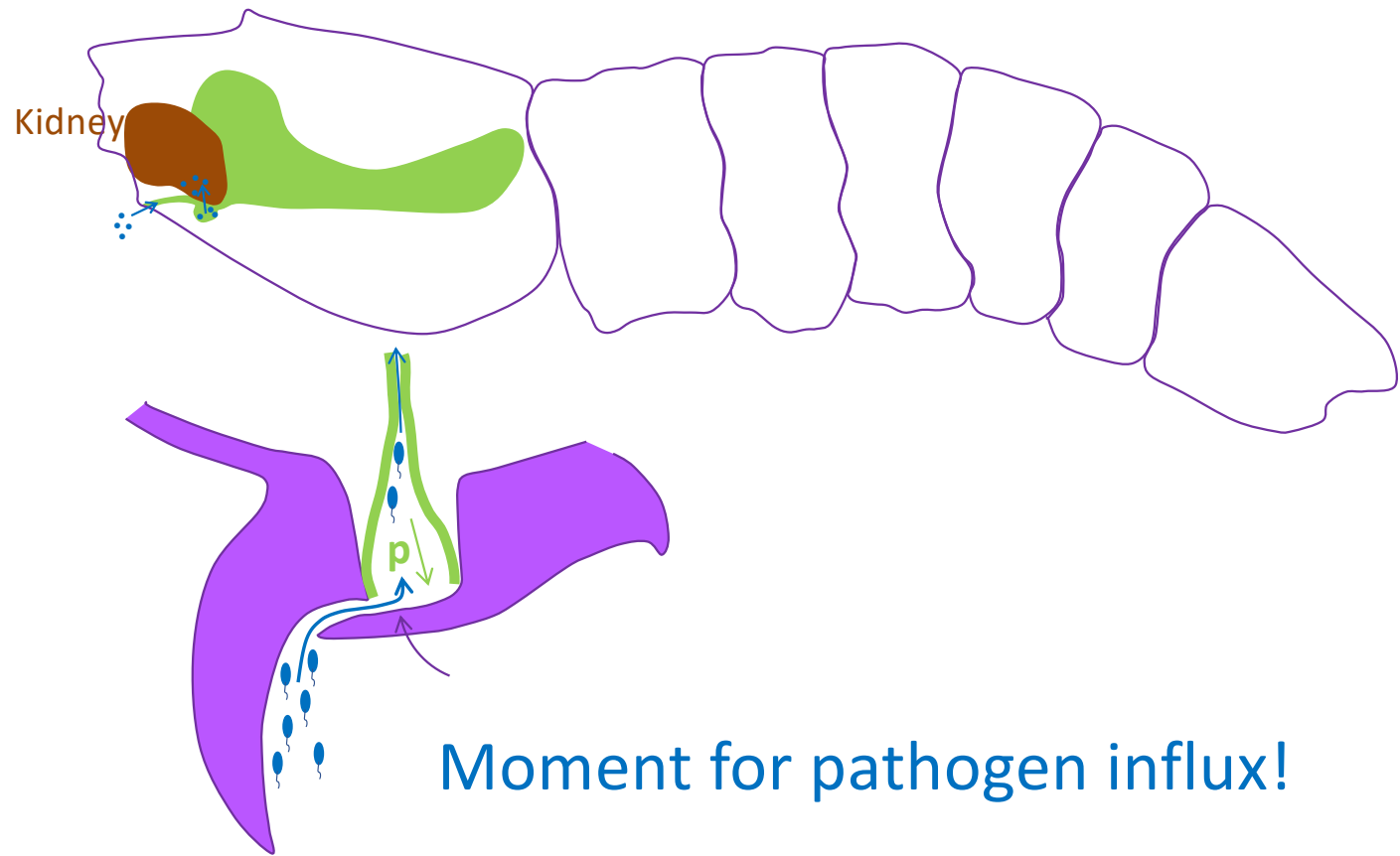


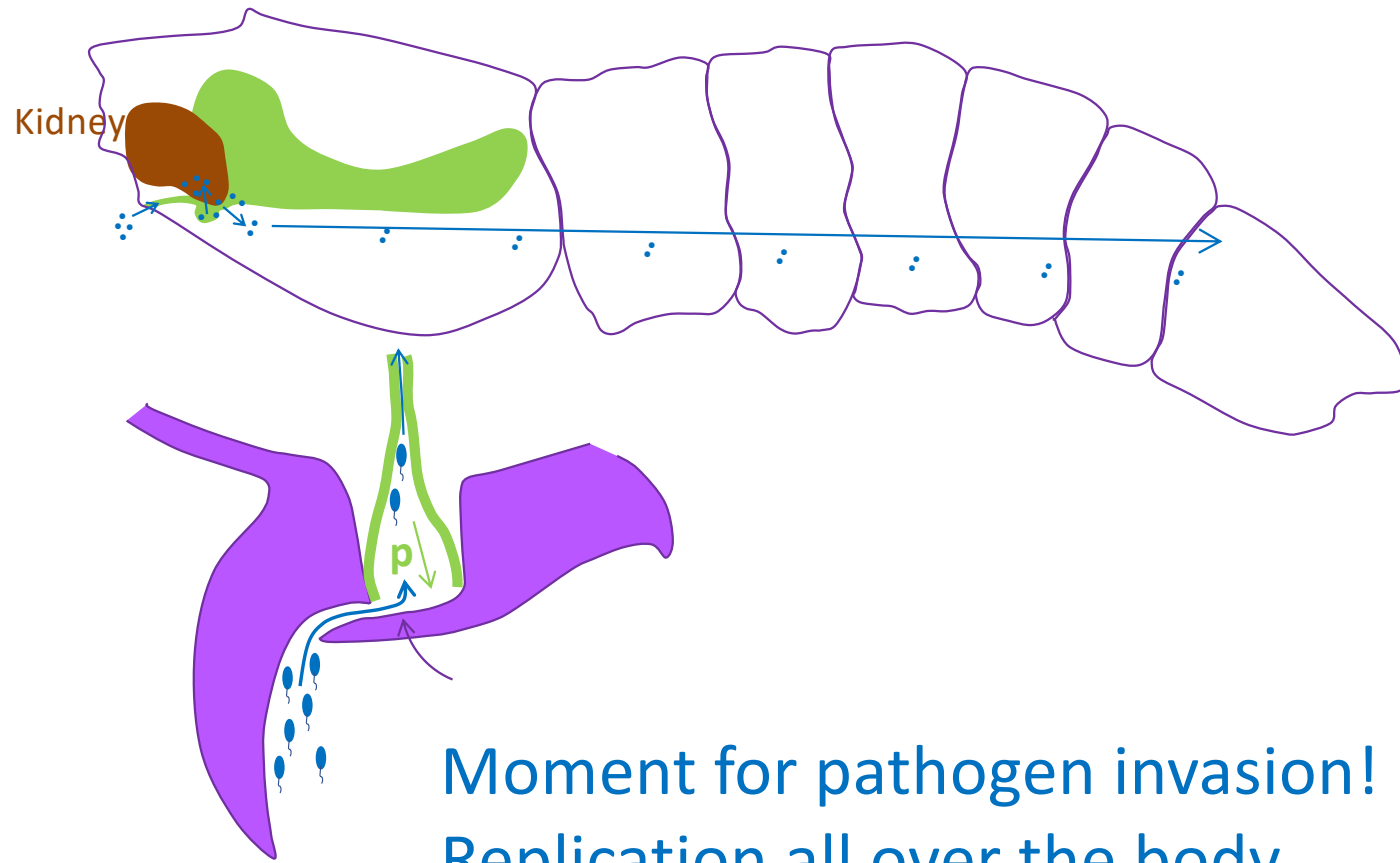
End of urination





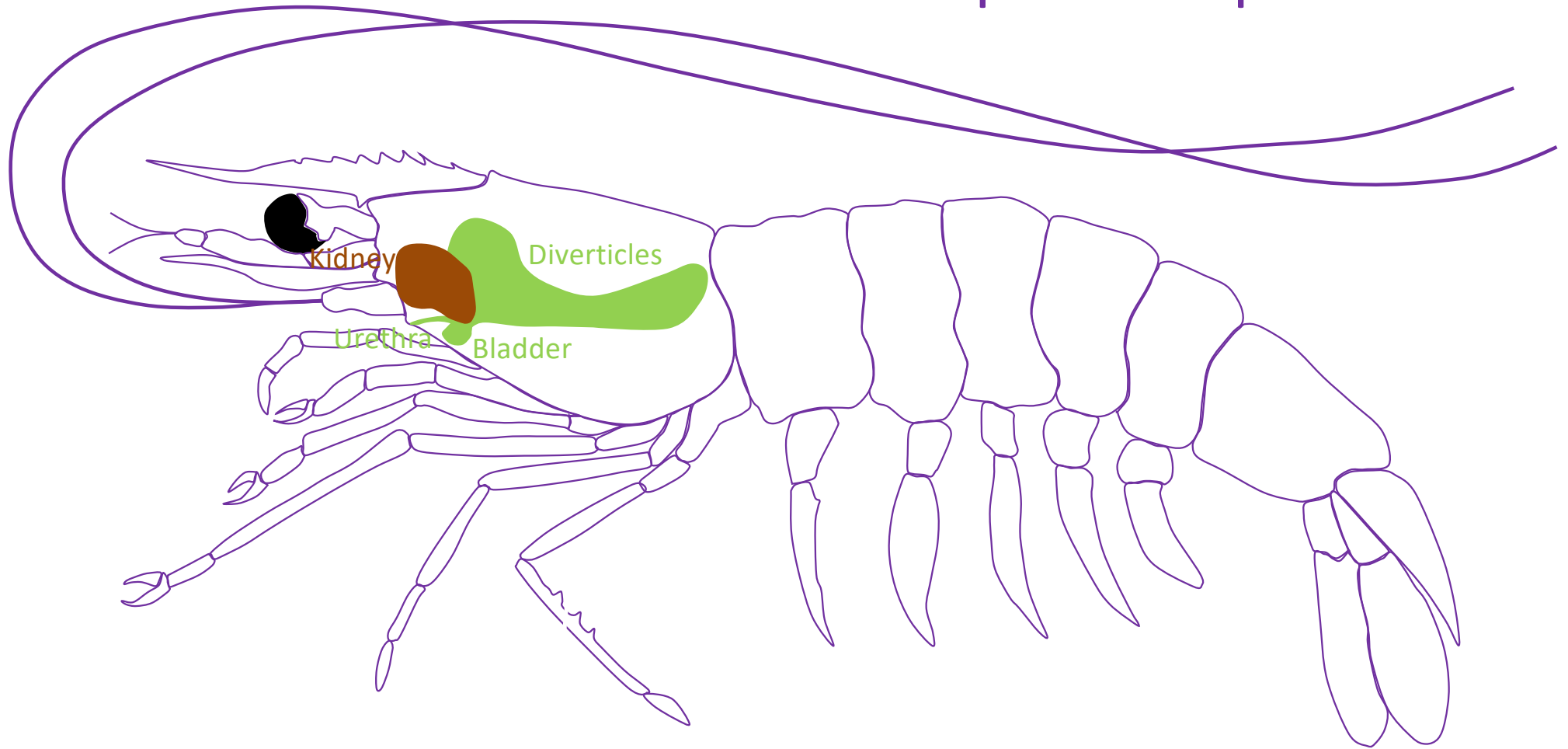
Moment for pathogen influx!
(experimentally demonstrated with beads)

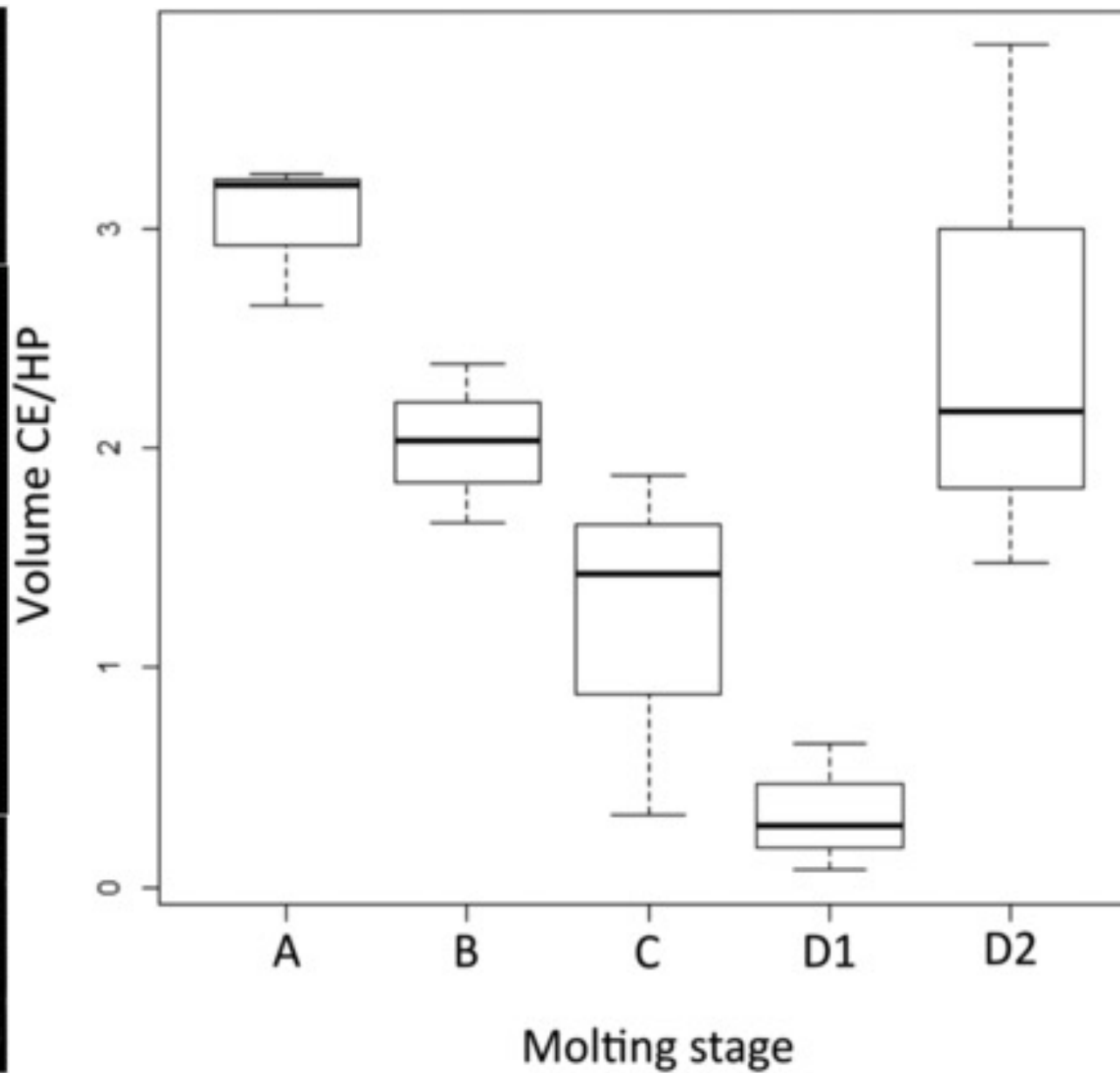
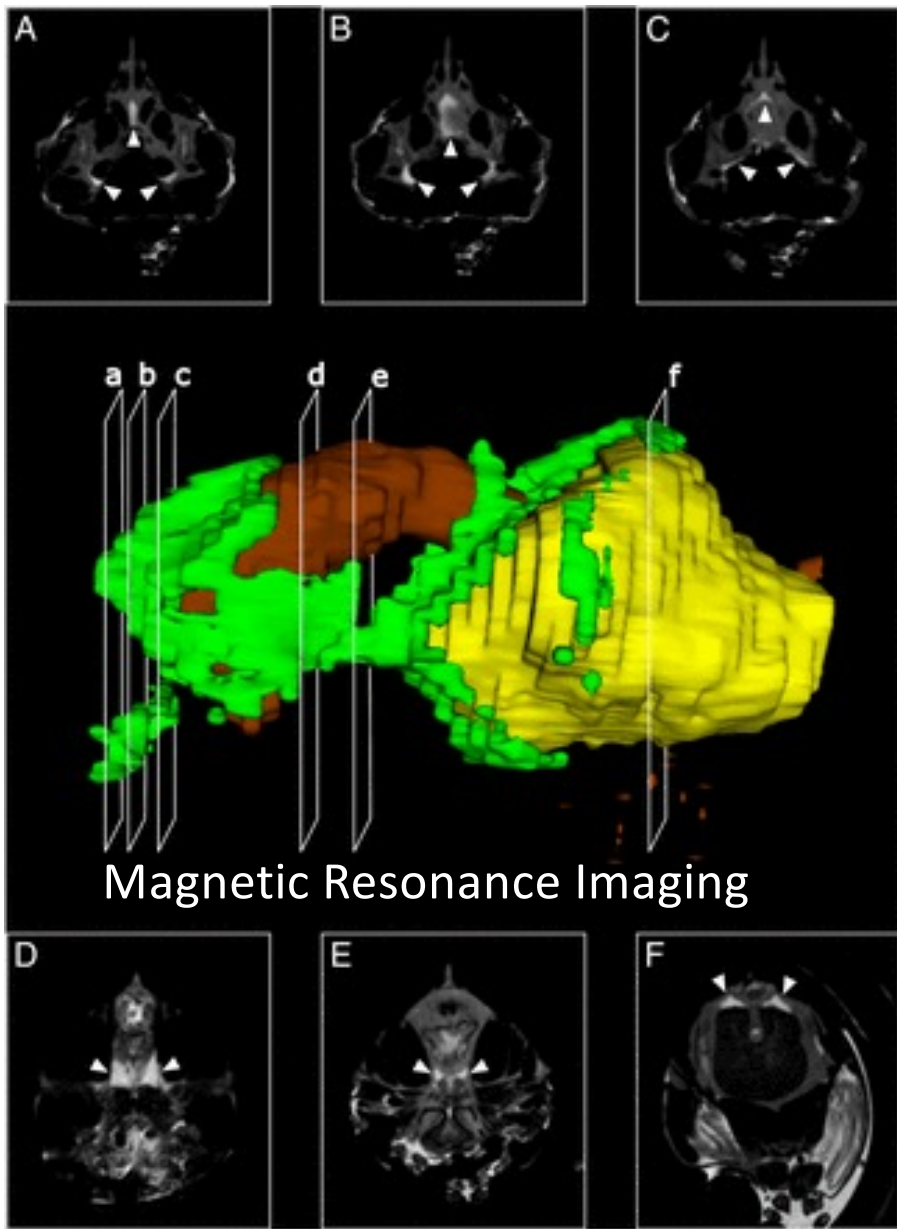




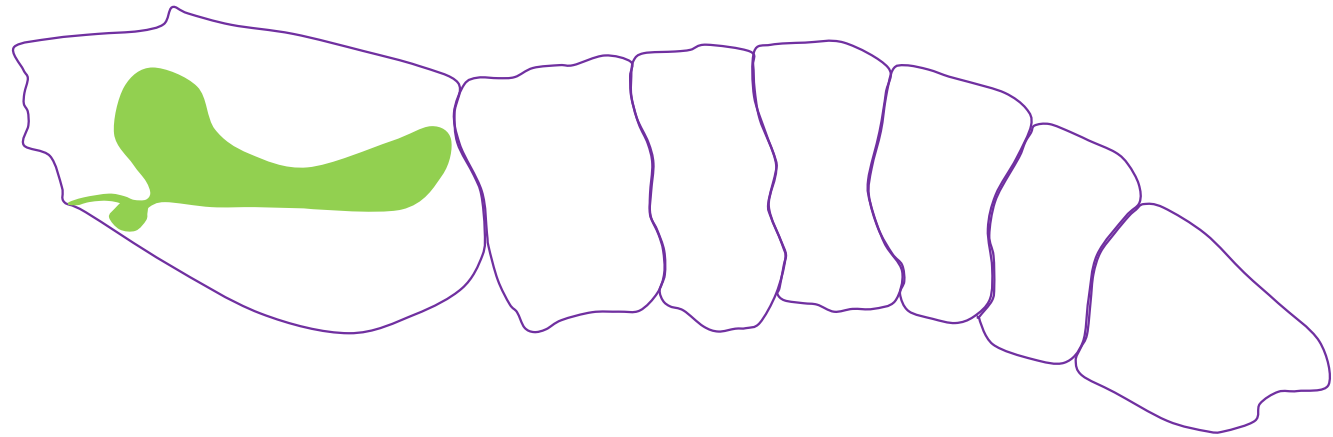
Moment for pathogen invasion!
Replication all over the body

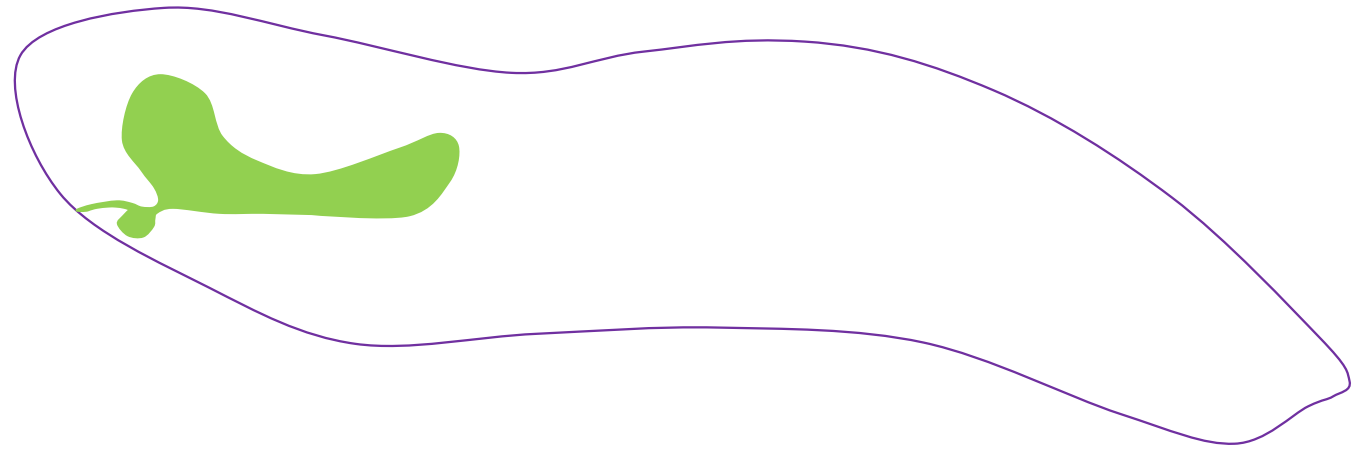
What is the function of the nephrocomplex?



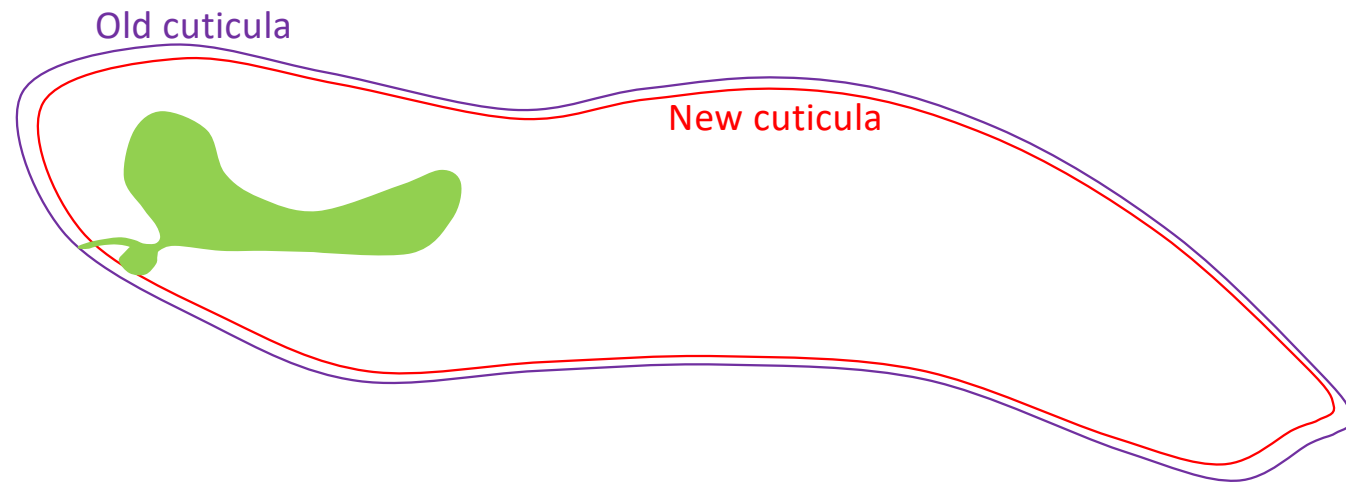


MODEL 2: ecdysis/molting

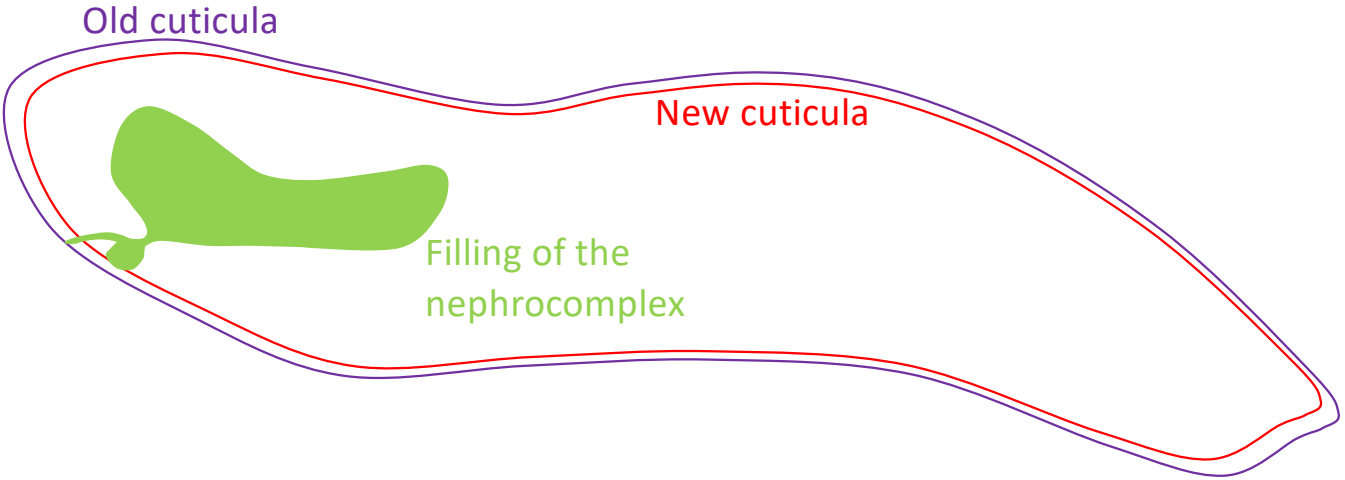




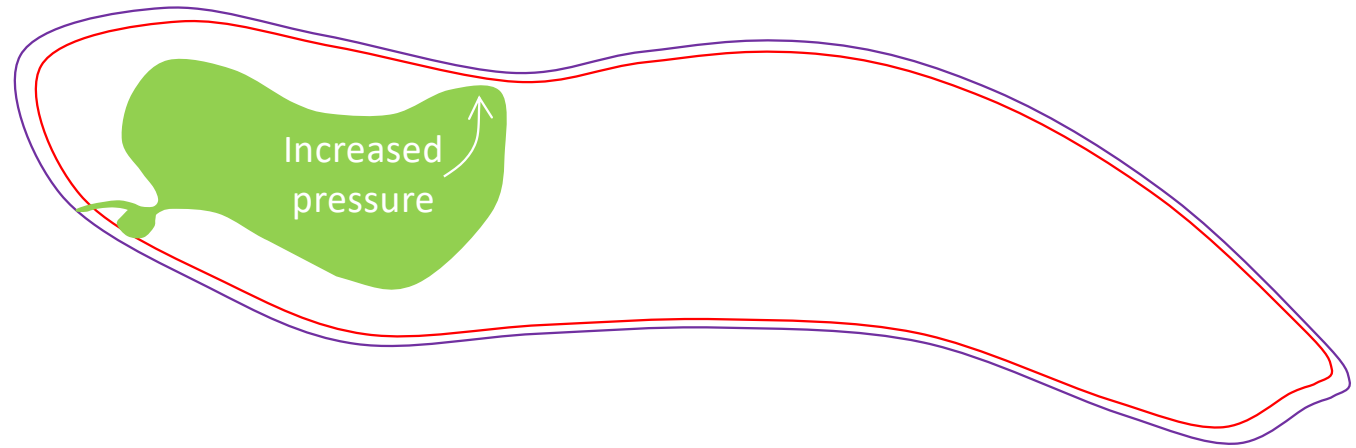
Before molting (stage D2)



Just before molting

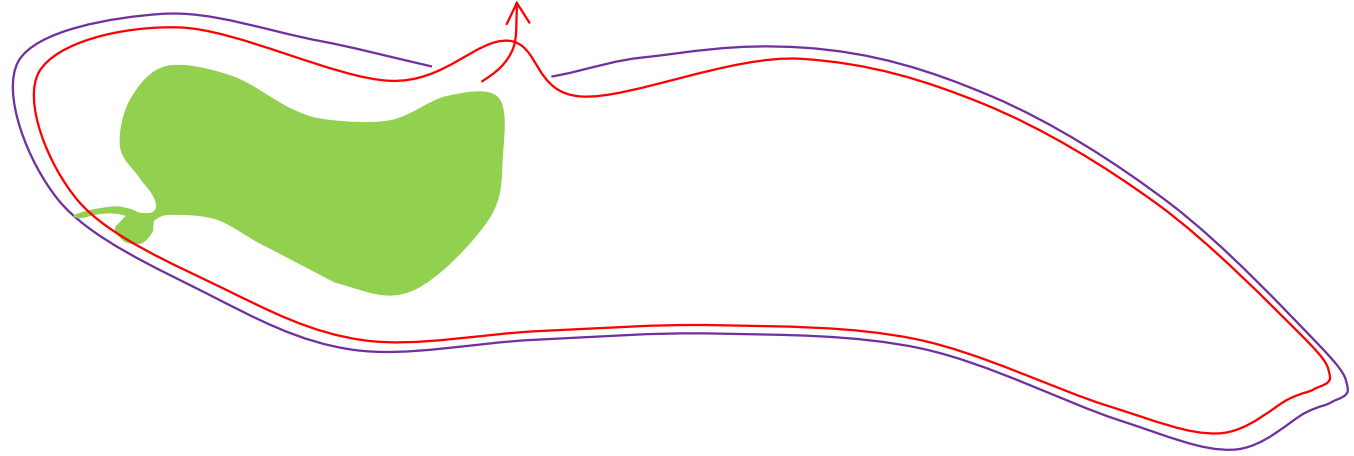


During molting

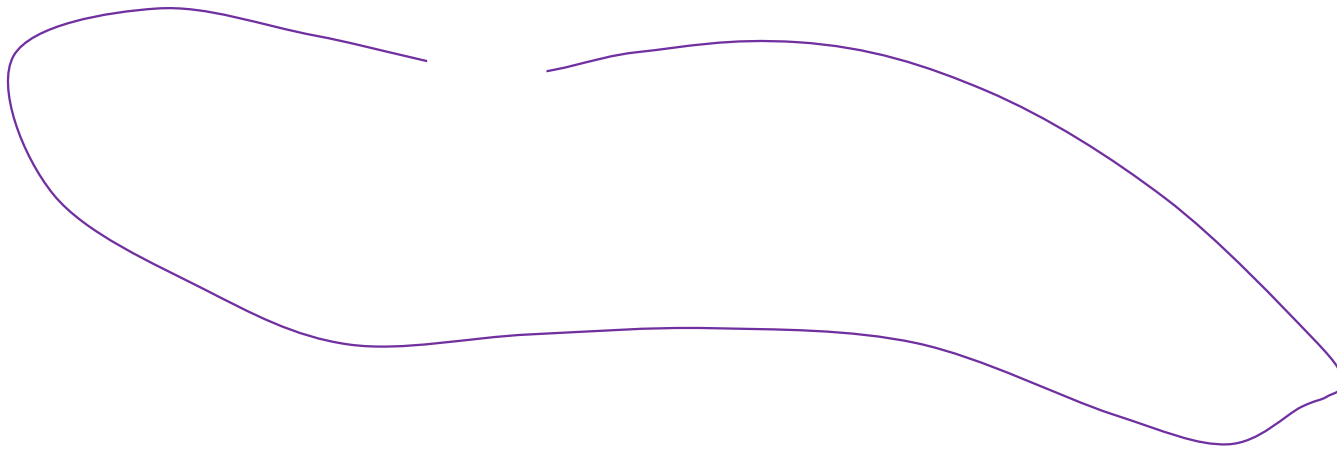
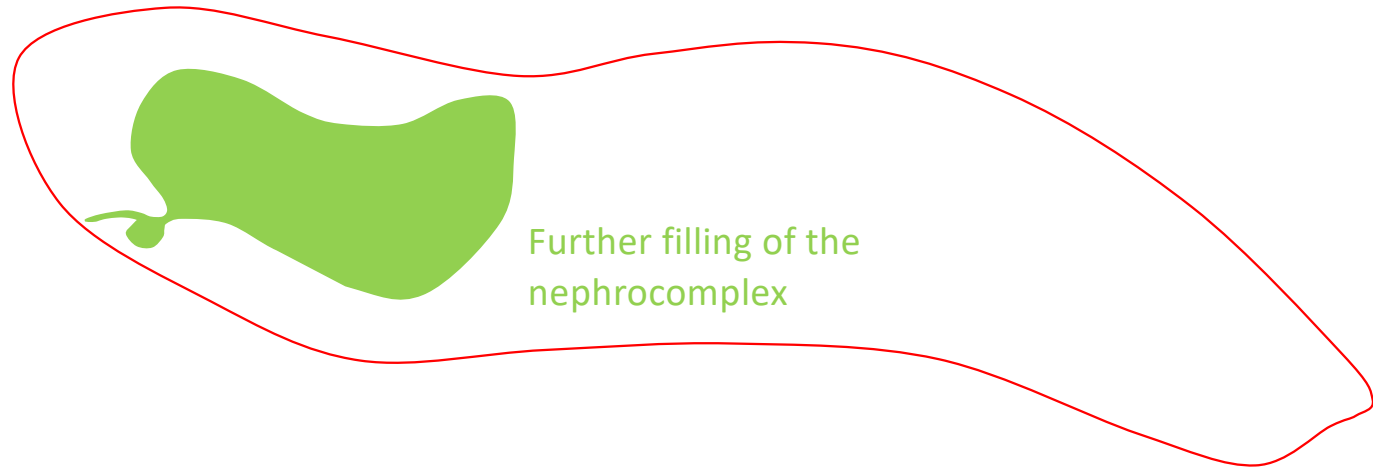


During molting

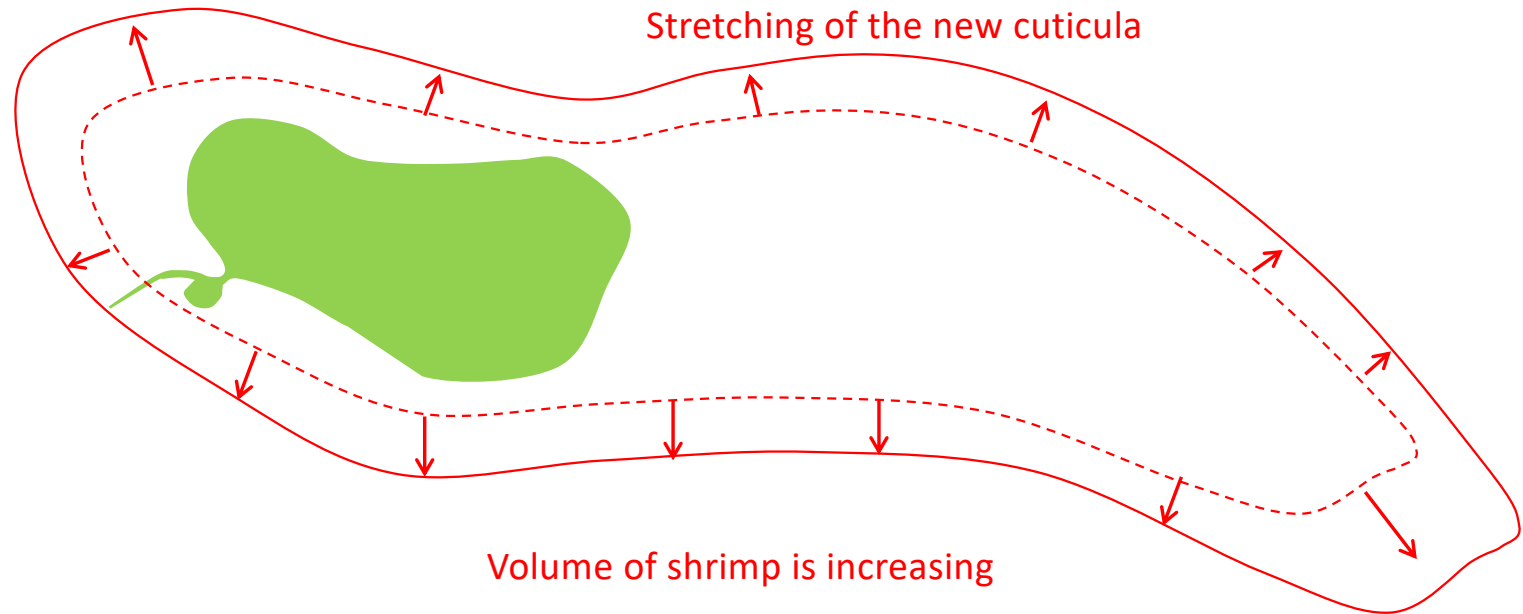
Rupture of the
old cuticle



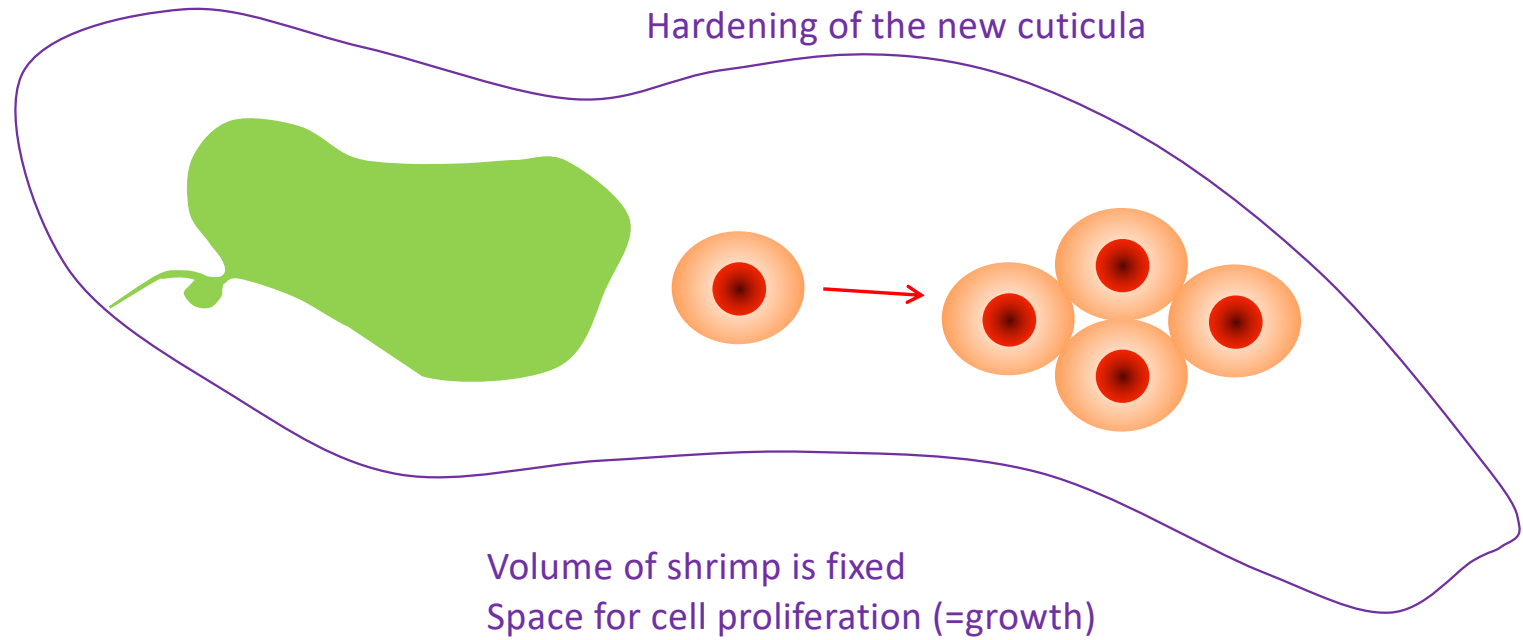
Just after molting (stage A)



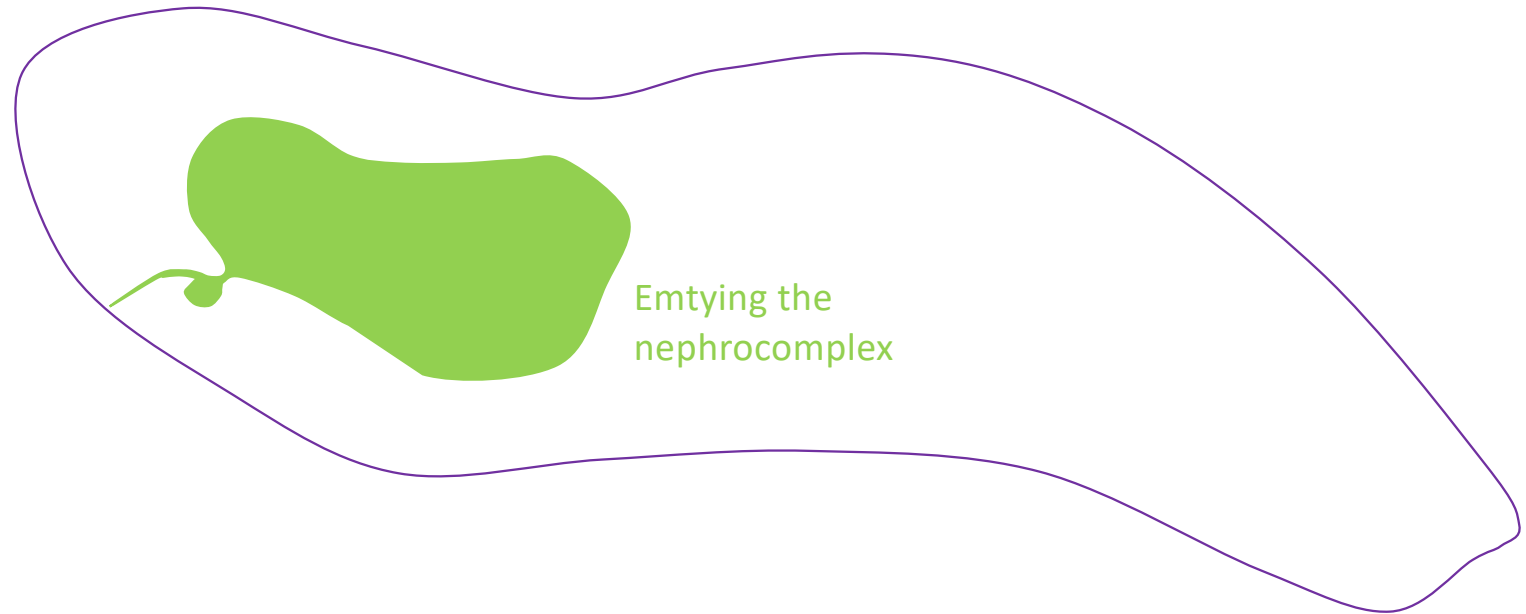
Just after molting (stage A)



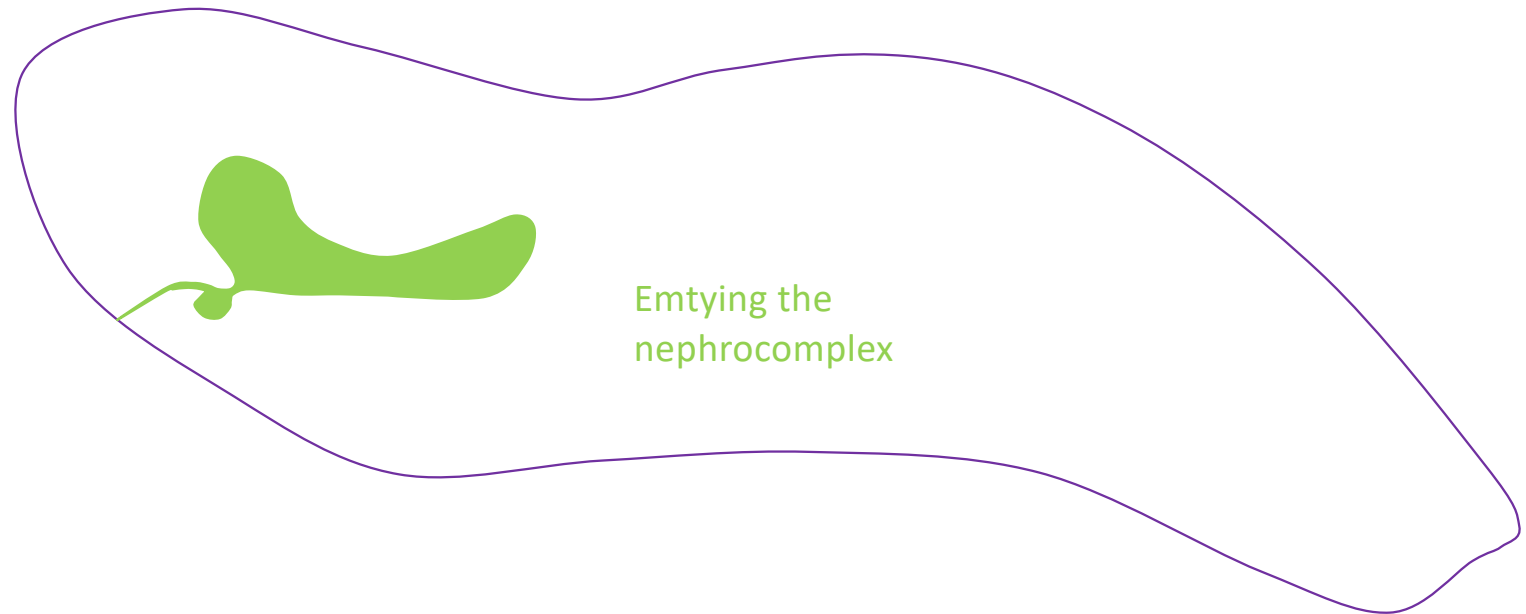
After molting (stage A)



After molting (stage A)



After molting (stages B-C-D1)



Everything is ready for the next molting