

Molt stage and cuticle damage influence waterborne WSSV entry in penaeid shrimp

Mathias Corteel, João Lima and Hans Nauwynck

Lab for Virology, fac Veterinary Medicine

June 12th 2008

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Penaeid shrimp

Penaeus monodon



Penaeus vannamei



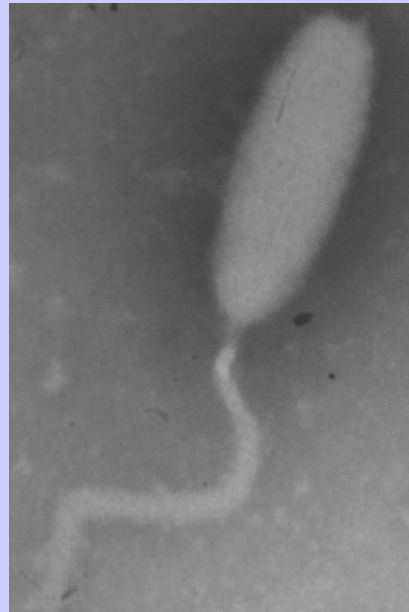
White Spot Syndrome Virus

family Nimaviridae

discovered begin '90s in S-E-Asia

ds DNA, circular 300 kbp

70-150 nm x 250-380 nm



low host specificity

quick spread!

envelop with 'tail'

end '90s all producing countries

White Spot Syndrome Virus

mass mortalities

upto 100% in 3-10 days



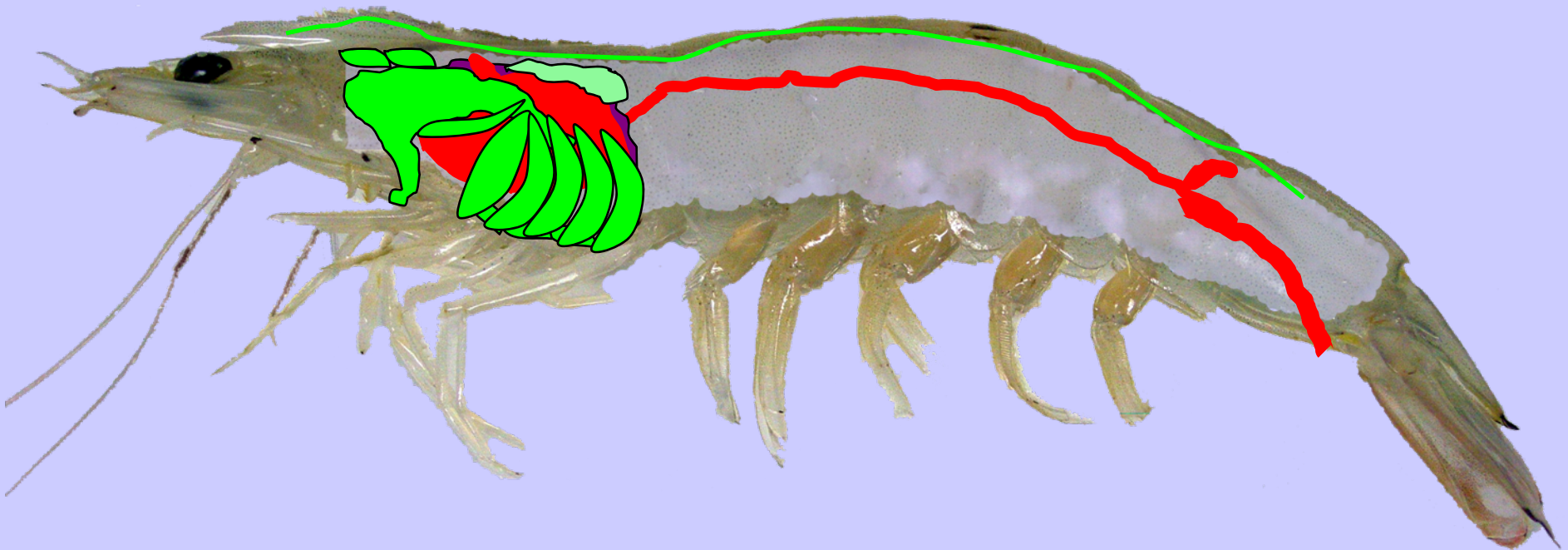
Transmission?

White Spot Syndrome Virus

Model of pathogenesis

primary replication: stomach, gills

secondary replication: internal organs, not endodermal tissues



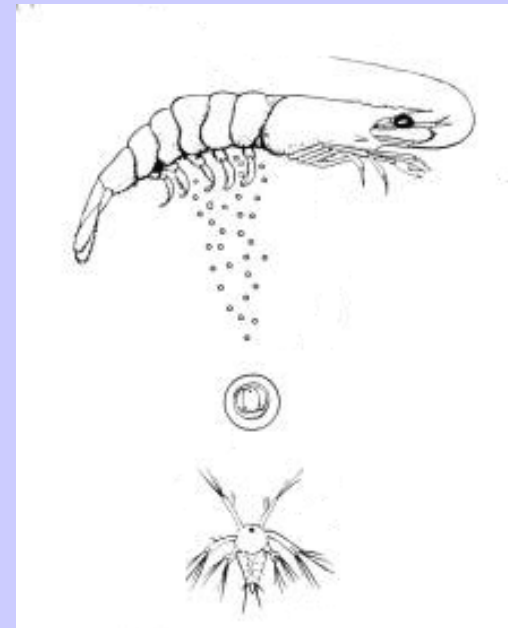
White Spot Syndrome Virus

Transmission route?

infected food: per os

infected water: waterborne

vertical ???

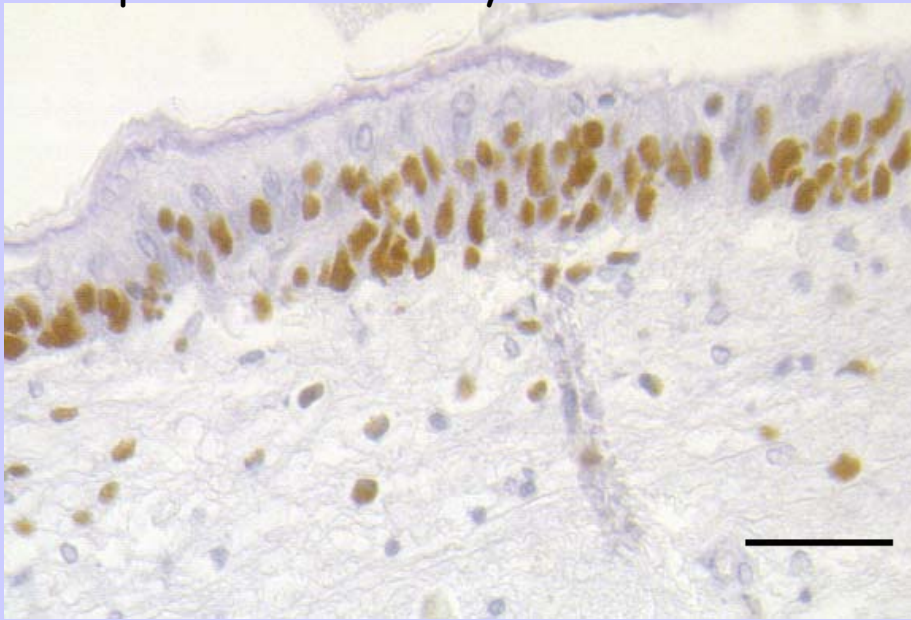


White Spot Syndrome Virus

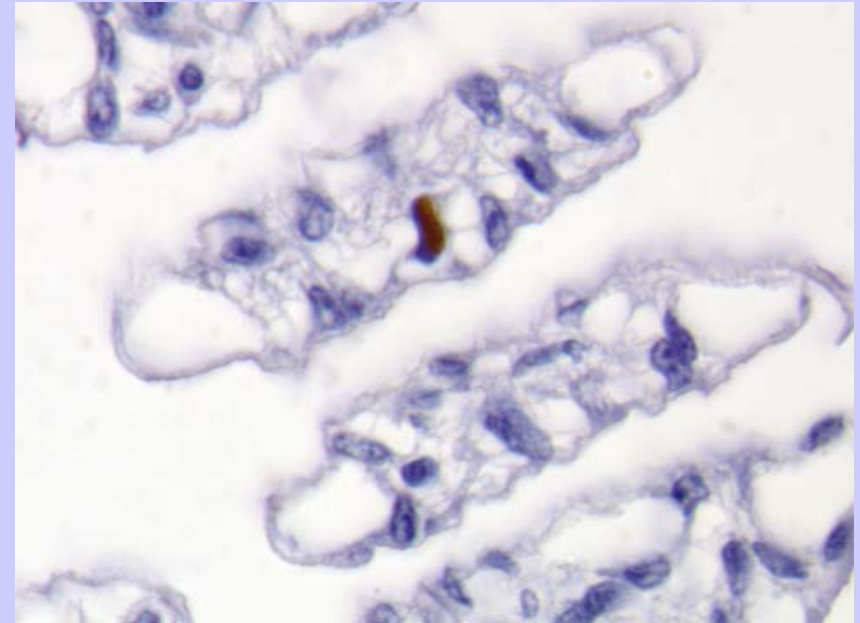
Transmission route?

target cells underneath cuticle!

epithelium of bodywall and stomach

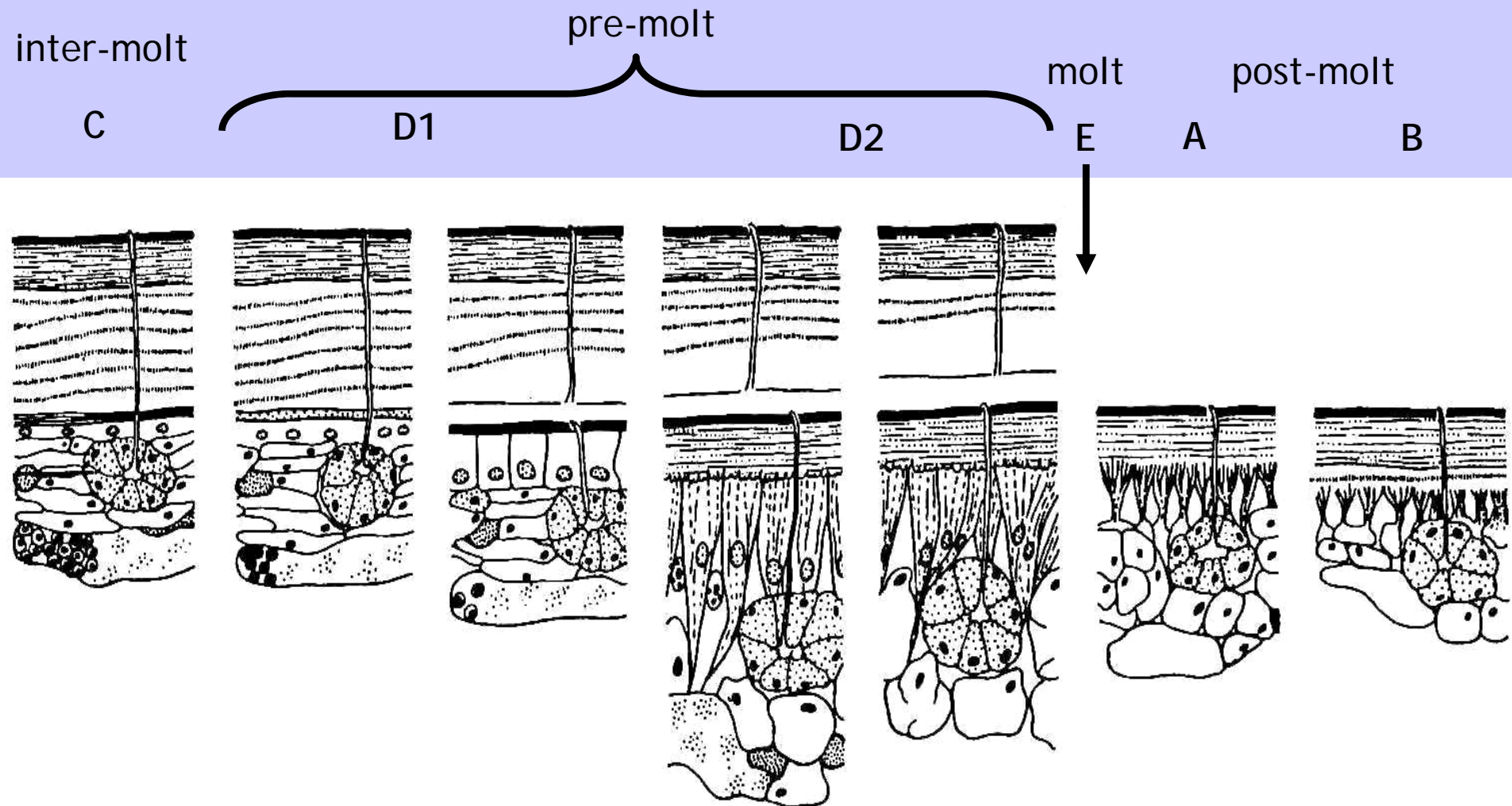


gills

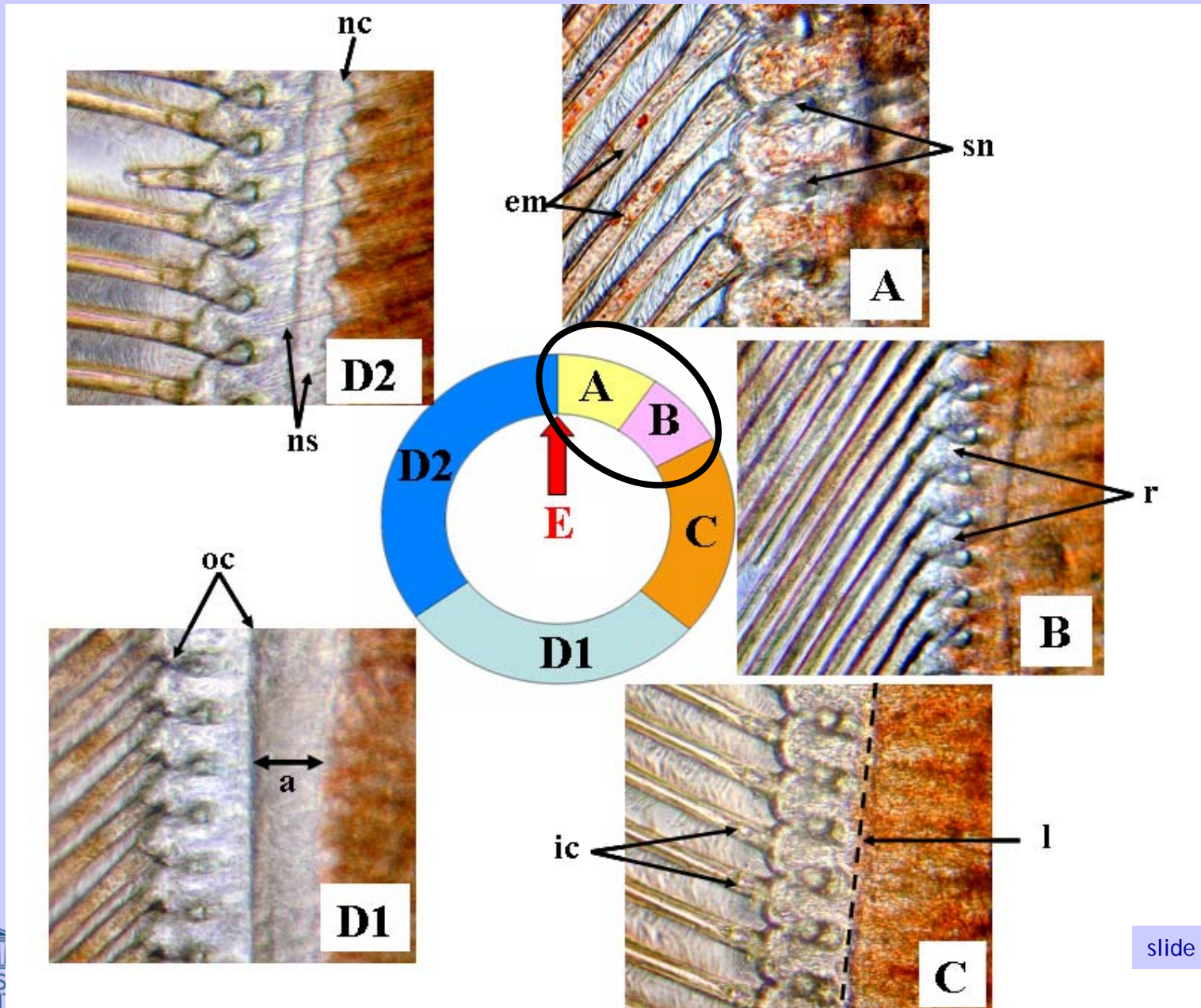


Cuticle barrier between target cells and virus inoculum?

cuticle = dynamic



Molt cycle



Cuticula barrier between target cells and WSSV in water?

→ immersion inoculation procedure

waterborne virus in literature

infection in some studies

very limited in others

→ influence of molt stage on susceptibility?

WSSV immersion inoculation in different stages of the molt cycle

1. Procedure with cell culture flasks:

- SPF shrimp *P. vannamei*
 - 5 groups:
 - A B C D1 D2
 - 5 shrimp each
- 3h

10000 SID₅₀ ml⁻¹

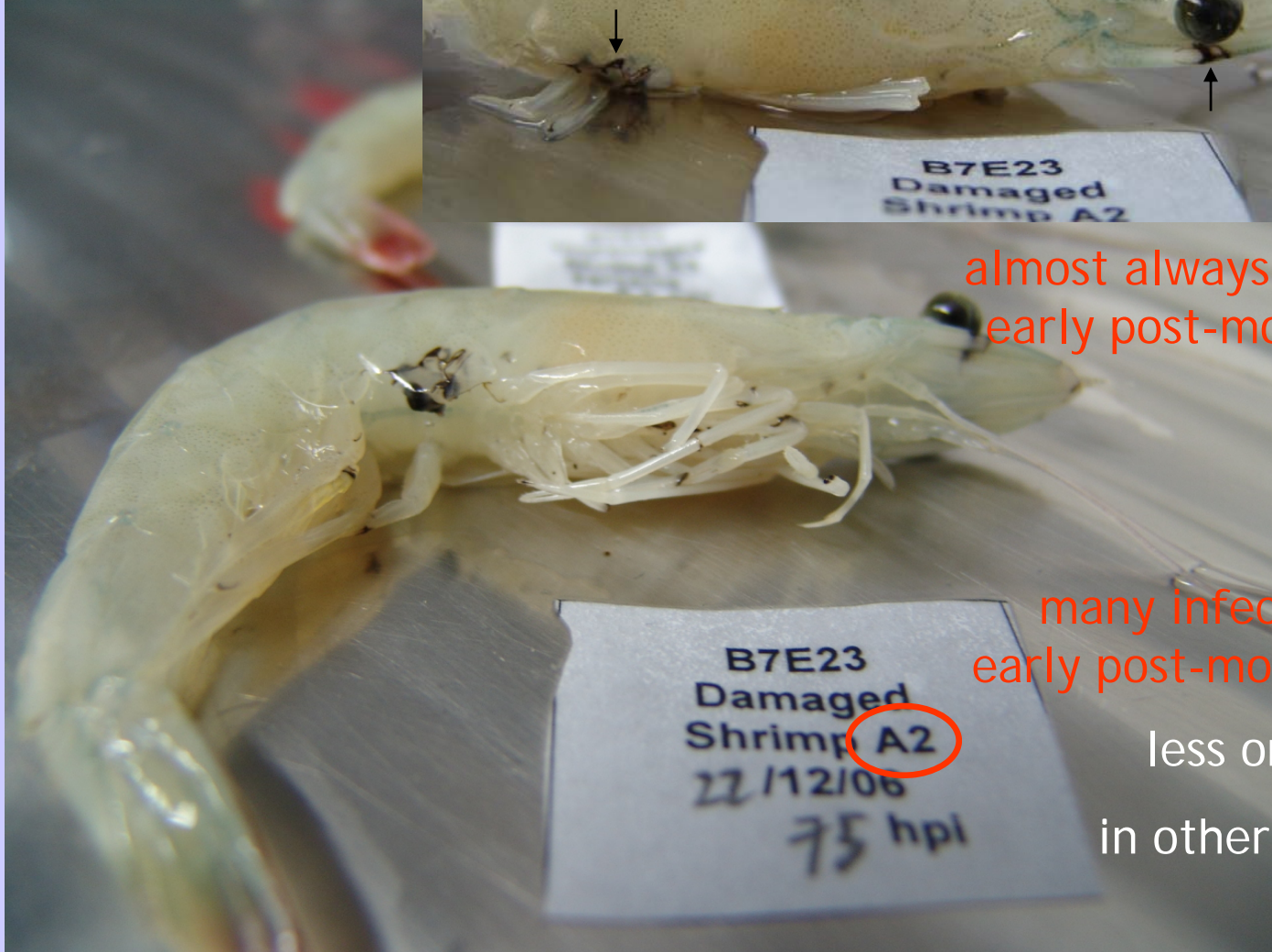


maintained individually for 5 days

monitor symptoms, mortality
infection status (IIF)

IM controls → all shrimp infected (A - D2) !

Weight	Molt stage	Mortality (hpi)	Results IIF
1 g	A	1/5 (60)	1/5 +
	B	0/5	0/5 +
	C	0/5	0/5 +
	D1	0/5	0/5 +
	D2	0/5	0/5 +
4 g	A	0/5	0/5 +
	B	0/5	0/5 +
	C	0/5	0/5 +
	D1	0/5	0/5 +
	D2	0/5	0/5 +
6 g	A	3/5 (60,60,84)	3/5 +
	B	0/5	0/5 +
	C	0/5	0/5 +
	D1	0/5	0/5 +
	D2	1/5 (<3)	0/5 +
11 g	A	5/5 (48,48,48,72,72)	5/5 +
	B	1/5 (120)	1/5 +
	C	0/5	0/5 +
	D1	0/5	0/5 +
	D2	0/5	0/5 +
20 g	A	5/5(48,60,60,60,72)	5/5 +
	B	3/5 (36,60,60)	2/5 +
	C	1/5 (60)	1/5 +
	D1	0/5	0/5 +
	D2	0/5	0/5 +



almost always damage in early post-molt A stage

many infected in early post-molt A stage

less or not in other stages

WSSV immersion inoculation in different stages of the molt cycle

2. Procedure with plastic bags, with/without damage



WSSV immersion inoculation in different stages of the molt cycle

2. Procedure with plastic bags, with/without damage

Aims:

induce (controlled) physical damage to the cuticula
by cutting pleopod

compare the difference in susceptibility to WSSV between
undamaged and damaged shrimp

for all the main stages of the molt cycle: A B C D1 D2

without induced damage

Molt stage	Shrimp	Damage		Mortality	IIF
		no pleopod cut of (-)	accidental		
A	1	-	rostrum and tail	+ (72)	+
	2	-	rostrum and tail	-	-
	3	-	rostrum	-	-
	4	-	-	-	-
	5	-	-	-	-
B	1	-	mutiple wounds	+ (72)	+
	2	-	-	-	-
	3	-	antennae	-	-
	4	-	-	-	-
	5	-	-	-	-
C	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
D1	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
D2	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-

with induced damage

Molt stage	shrimp	Damage		Mortality	IIF
		1 pleopod cut of (+)	accidental		
A	1	+	-	+ (48)	+
	2	+	mutiple wounds	+ (72)	+
	3	+	rostrum en tail	+ (84)	+
	4	+	tail	+ (84)	+
	5	+	rostrum	+ (120)	+
B	1	+	-	+ (96)	+
	2	+	-	+ (96)	+
	3	+	-	-	-
	4	+	pereiopods	-	-
	5	+	-	-	-
C	1	+	-	+ (120)	+
	2	+	-	-	-
	3	+	-	-	-
	4	+	-	-	-
	5	+	-	-	-
D1	1	+	-	-	-
	2	+	-	-	-
	3	+	-	-	-
	4	+	-	-	-
	5	+	-	-	-
D2	1	+	-	-	-
	2	+	-	-	-
	3	+	-	-	-
	4	+	-	-	-
	5	+	-	-	-

WSSV immersion inoculation in different stages of the molt cycle

repeated in 2 sizes of shrimp
2 shrimp species
2 locations of damage

(total +300 shrimp)

ALWAYS:

1. more infection in post-molt stages and inter-molt **window?**
2. no infection in pre-molt **defence?**
3. 2 - 8 X more infection when damage was induced **natural?**

Further research on WSSV transmission

locate primary site of entry + replication

compare clotting time of hemolymph
and structure of clot

between different molt stages

investigate role of hemocytes in infection

compare structure of cuticle + epidermis
between different molt stages

use other mechanical techniques to damage cuticle

replace mechanical damage by
bacteria or fungi to colonize and digest cuticle

Other research

Culture of shrimp cells and explants in vitro

Macrobrachium rosenbergii and WSSV

Product and susceptibility testing for companies

Yellow head virus

Antiviral defence of shrimp

Thank you



Laboratory for Virology