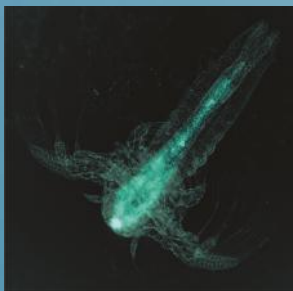
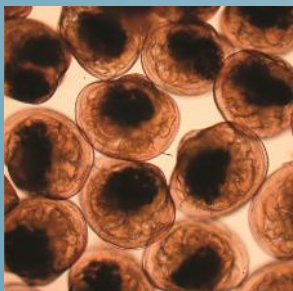
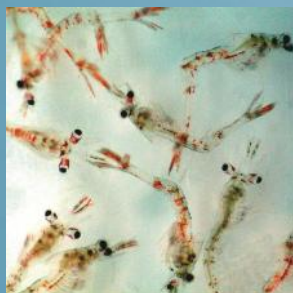
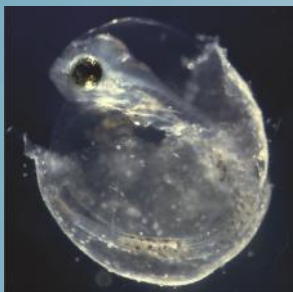


larvi 2013

6th fish & shellfish larviculture symposium



Inducers of heat shock protein 70:
A new disease preventive option in
aquaculture production systems

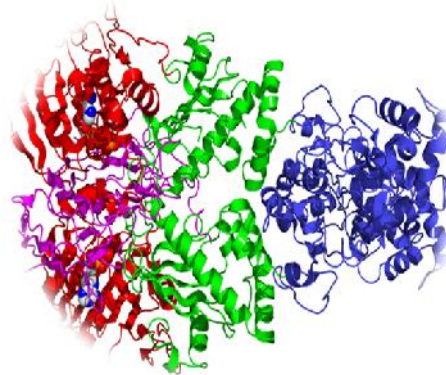
Kartik Baruah



ghent university, belgium, 2-5 september 2013



Inducer of Heat Shock Protein 70: A New Disease Preventive Option in Aquaculture Production Systems

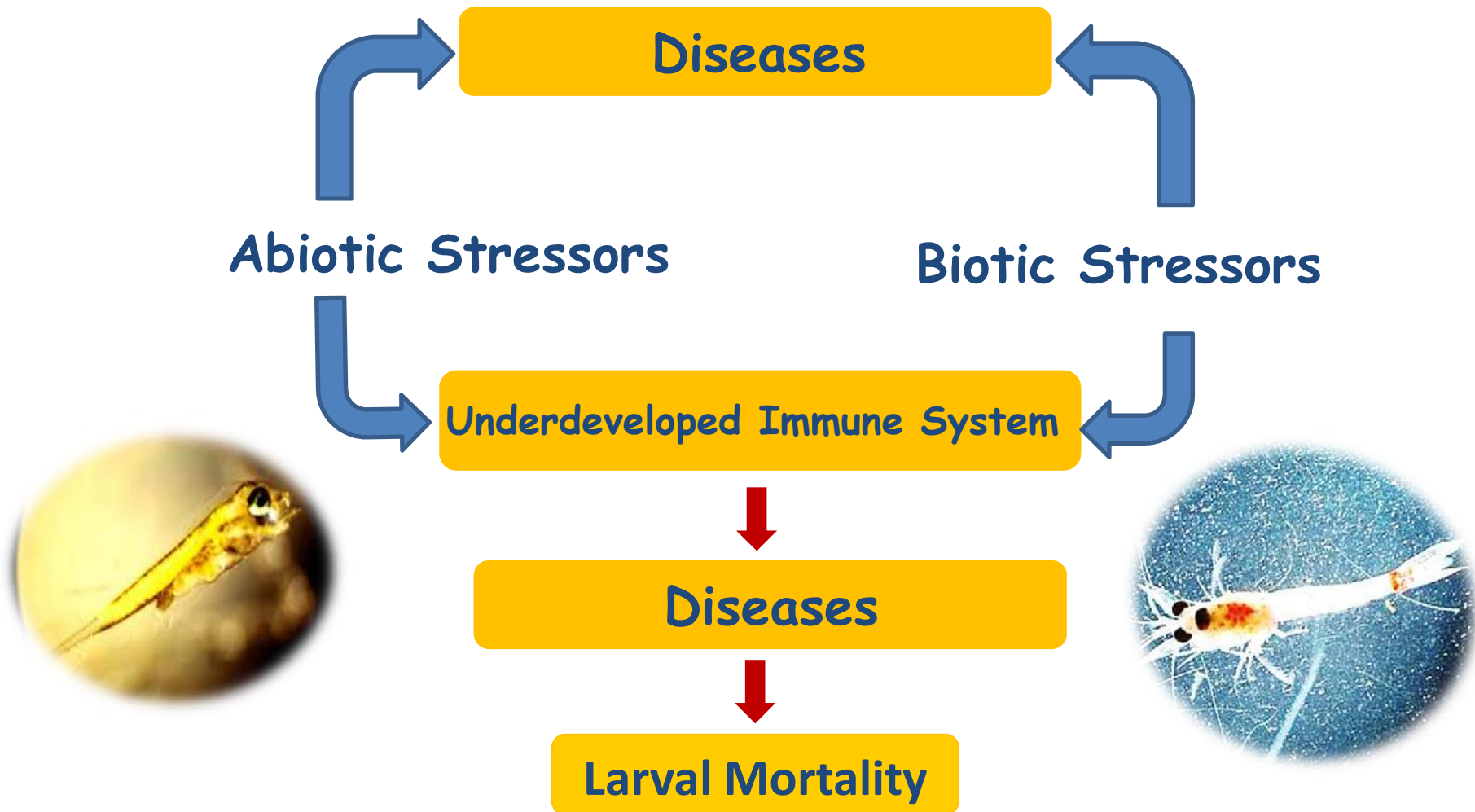


Dr. Kartik Baruah

Parisa Norouzitallab, Patrick Sorgeloos & Peter Bossier



Bacterial diseases – Still a Threat to Larviculture



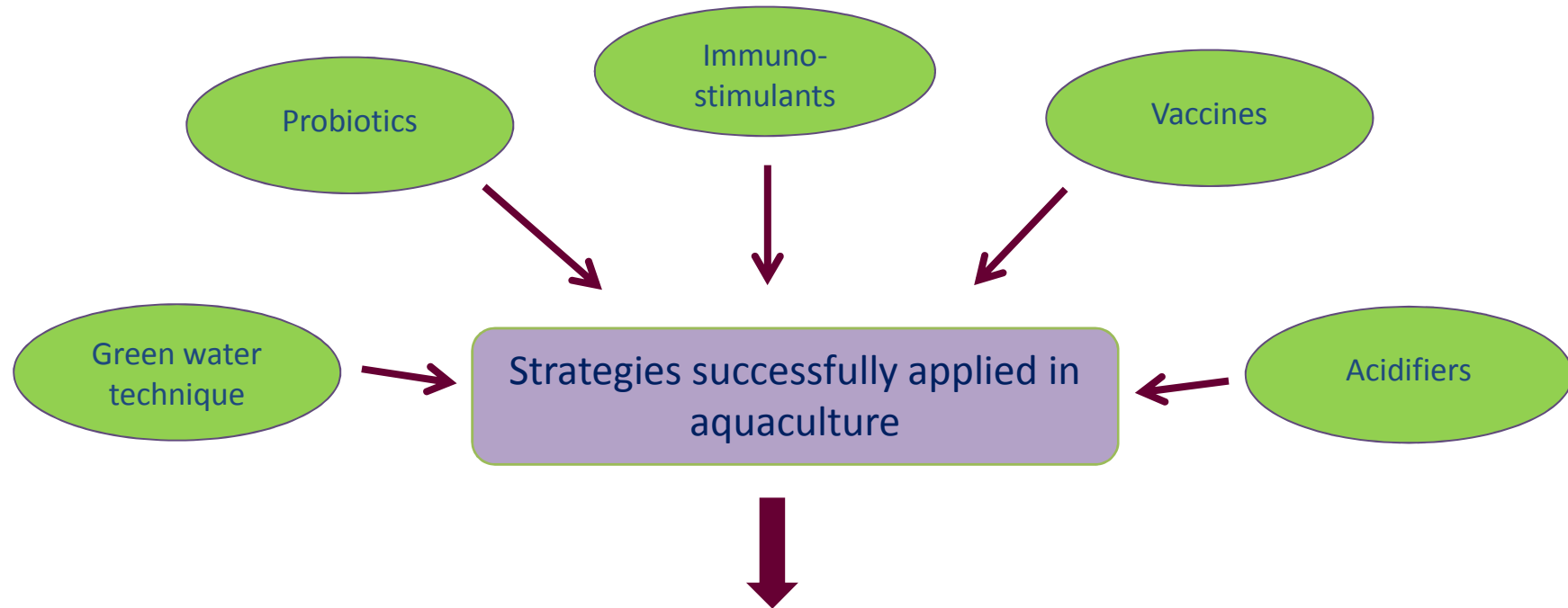
Antibiotics – The Unsustainable Solution



Emphasis on **PREVENTION** which is likely to be more cost effective than the **CURE**

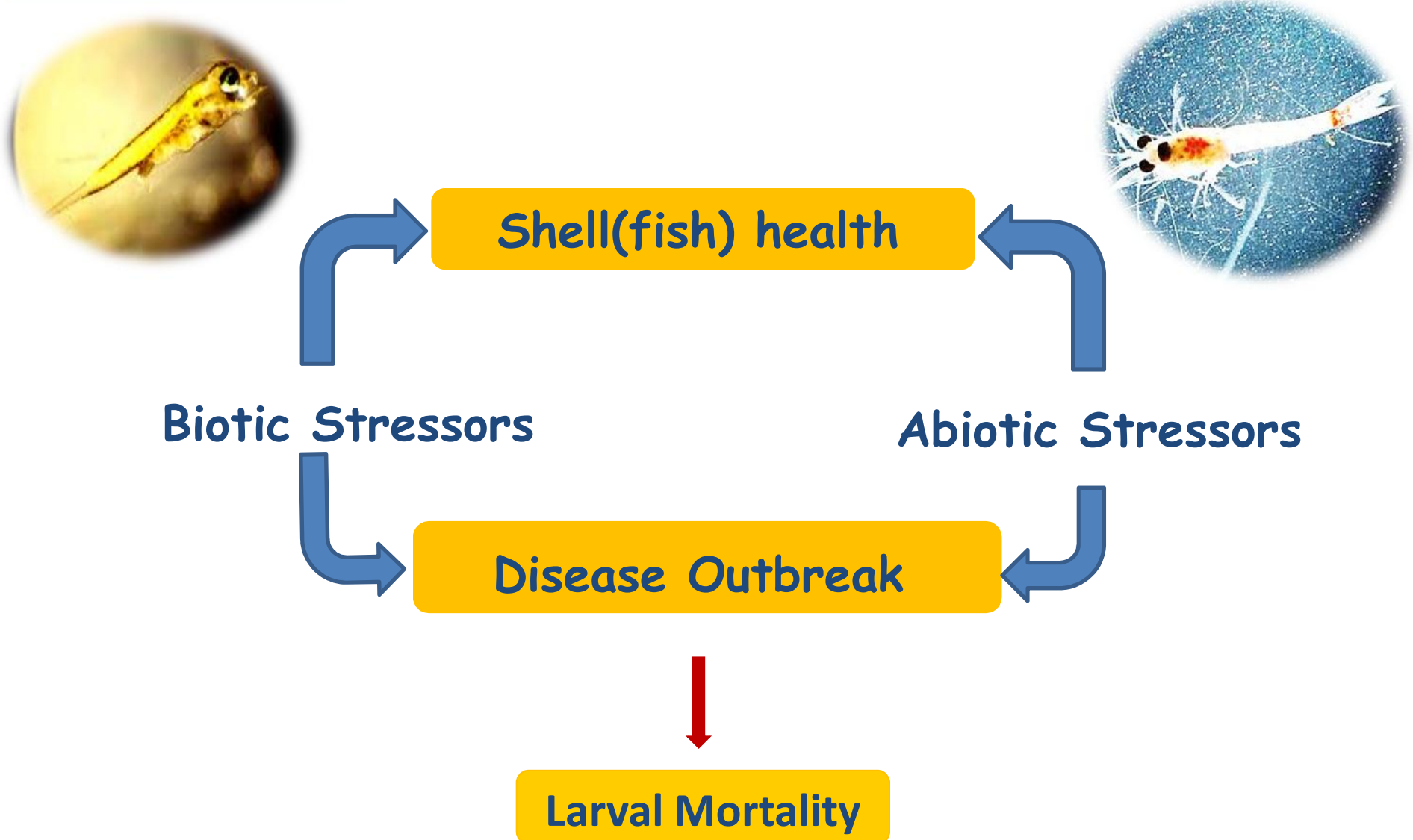


Alternative Solutions for Larval Diseases



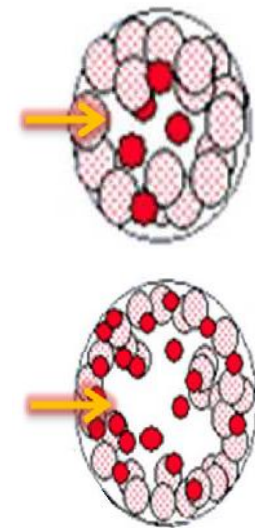
More alternatives required as no anti-infective technique seems to be able to solve every problem alone.

Multiple agents cause disease outbreak



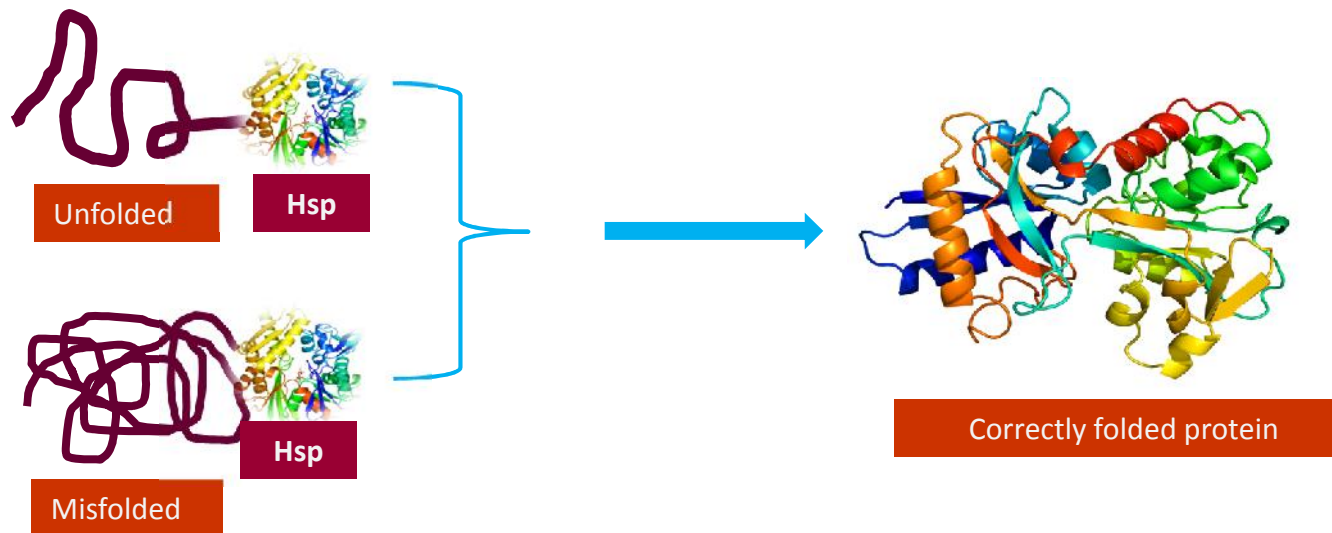
What are Heat Shock Proteins (Hsps)?

- Hsps are ubiquitous and highly conserved protein molecules, available in all prokaryotic and eukaryotic cells.
- Different types – sHsps, Hsp60, Hsp70, Hsp90, Hsp100.
- Hsp70s form one of the major Hsp families, most extensively studied one.
- Synthesized constitutively in the cells (heat shock cognate 70).
- Induced after exposure to stressors (cold, O₂ deprivation, etc).



Function of Intracellular Hsp

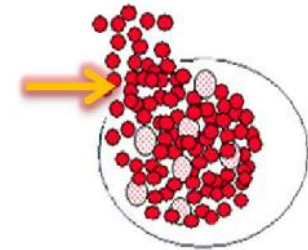
- Inside the cell they act as molecular chaperones - bind to unfolded proteins (nascent polypeptides or denatured ones) - facilitate their refolding to the native state.



- Involved in protein translocation and degradation.

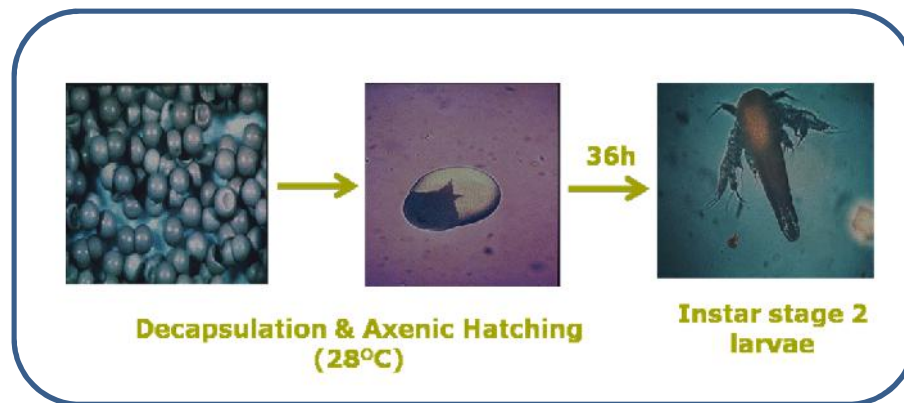
Function of Extracellular Hsp

- Due to necrosis, the Hsp70 gets released from the cell.

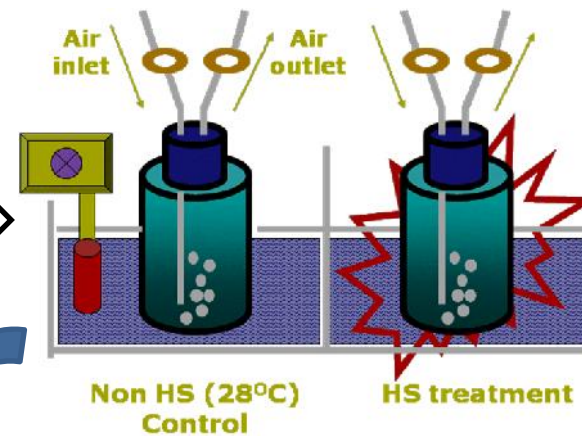


- Extracellular Hsp70 serves as danger signal and modulates both innate and adaptive immune responses.

Hsp70 Induction in *Artemia* : Non-Lethal Heat Shock (NLHS)



Gnotobiotic *Artemia*



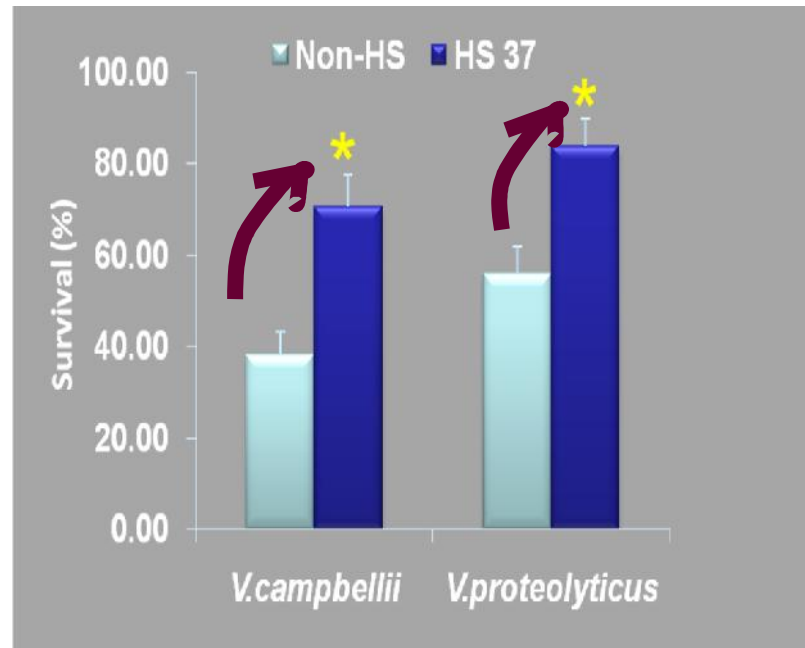
NLHS at 37°C for 30 min followed by 6 h recovery period

Challenged with vibrios at 10^7 cells/ml

Survival scored after 48 h

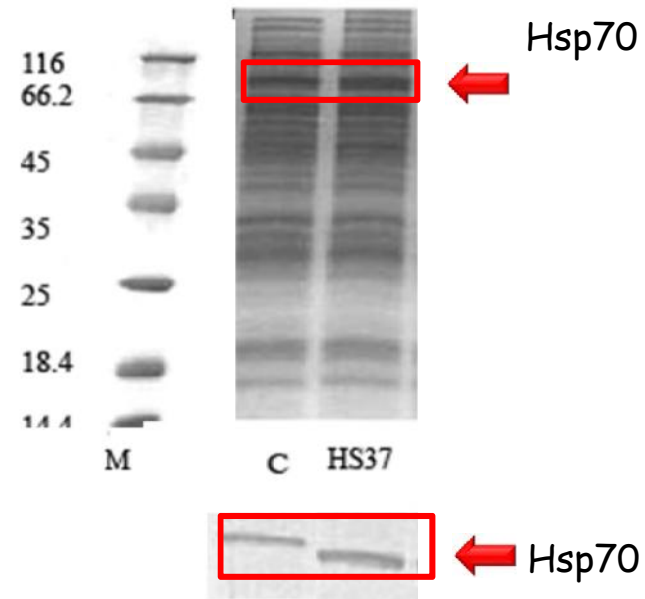
Results

Survival after *Vibrio* challenge



vs

Endogenous Hsp70 accumulation



- Correlation exists between enhanced protection and Hsp70 accumulation

QUESTION ????

- Heat shock is not an ideal way to enhance Hsps in aquaculture animals.
- Is there any less traumatic approach to manipulate Hsps expression within (shell)fish?

Compounds extracted from plants



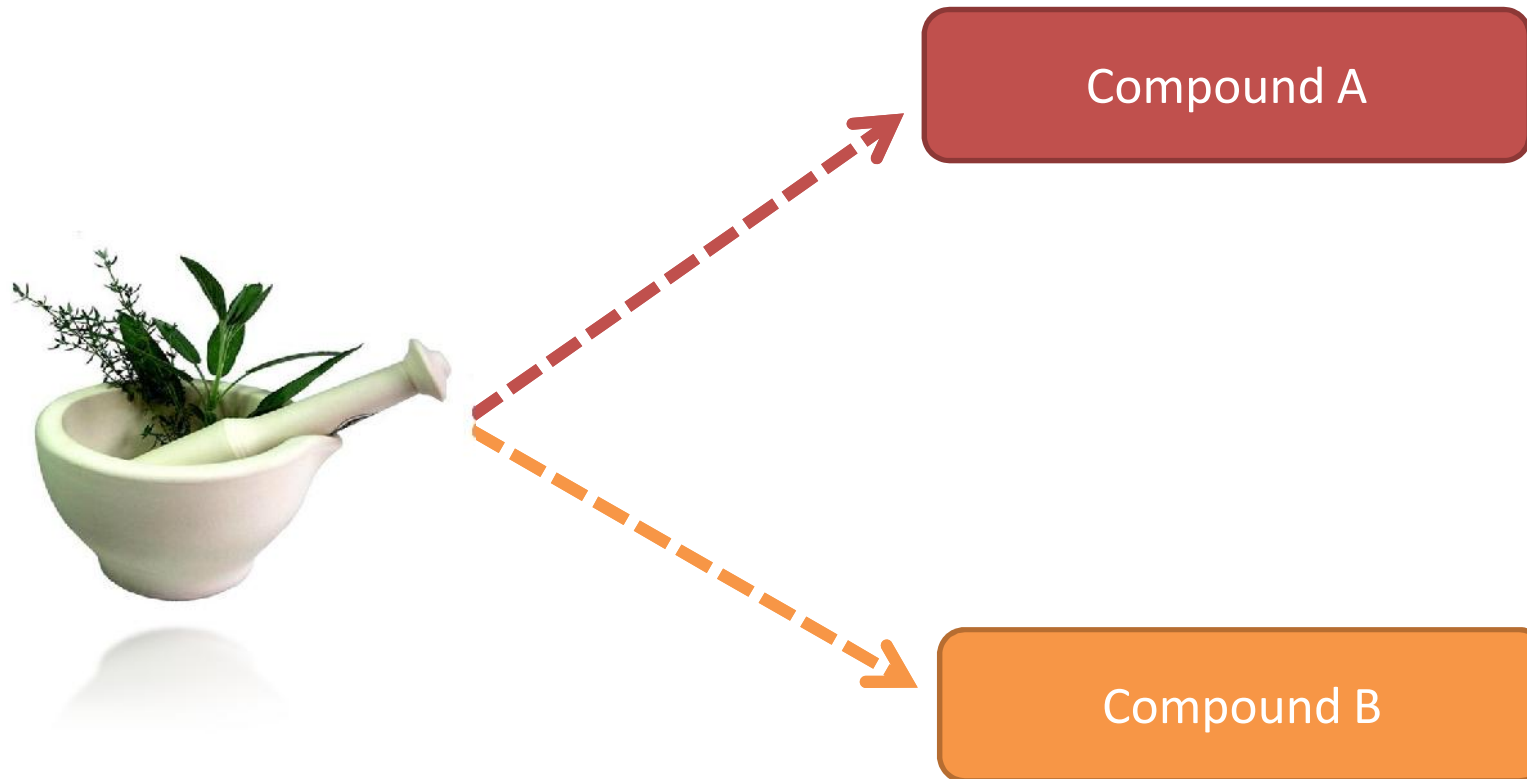
Can they induce Hsp70 production in aquaculture animals???

Can they induce protection against abiotic stressors???

Can they induce protection against pathogenic biotic stressors???

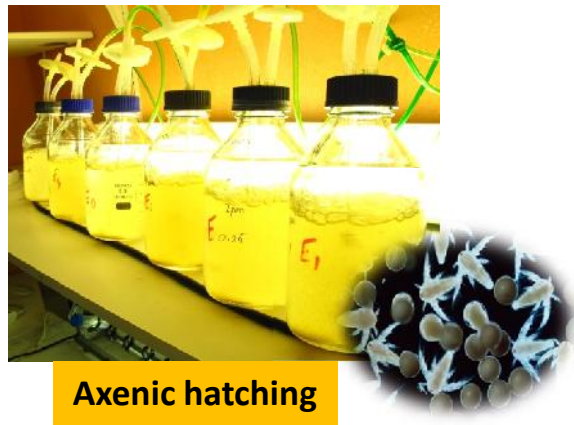


Compounds extracted from plants



- Unpublished work in progress

Pretreatment of axenic *Artemia* with compound



Pretreatment of *Artemia* instar II with compound for 2 h (28°C)

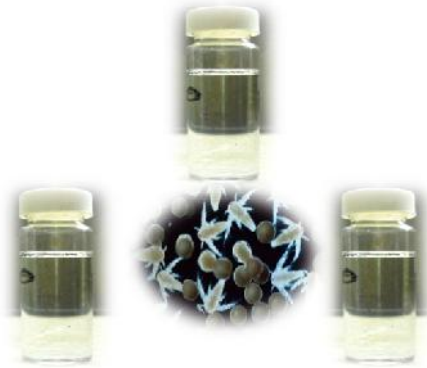
Washing of *Artemia* with sterile sea water (28°C)

Given a recovery period of 2 h (28°C)



Challenge with abiotic or pathogenic biotic stress

Thermal stress

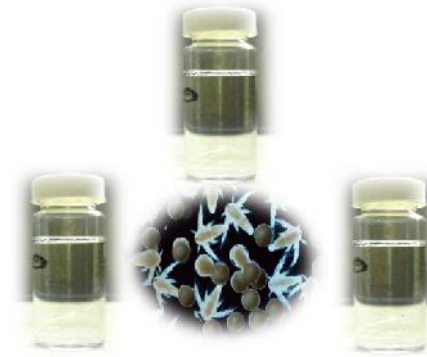


Heat shock at 41°C for 20 min



Survival scored after 6 h

Vibrio challenge



Challenged at 10^7 cells/mL

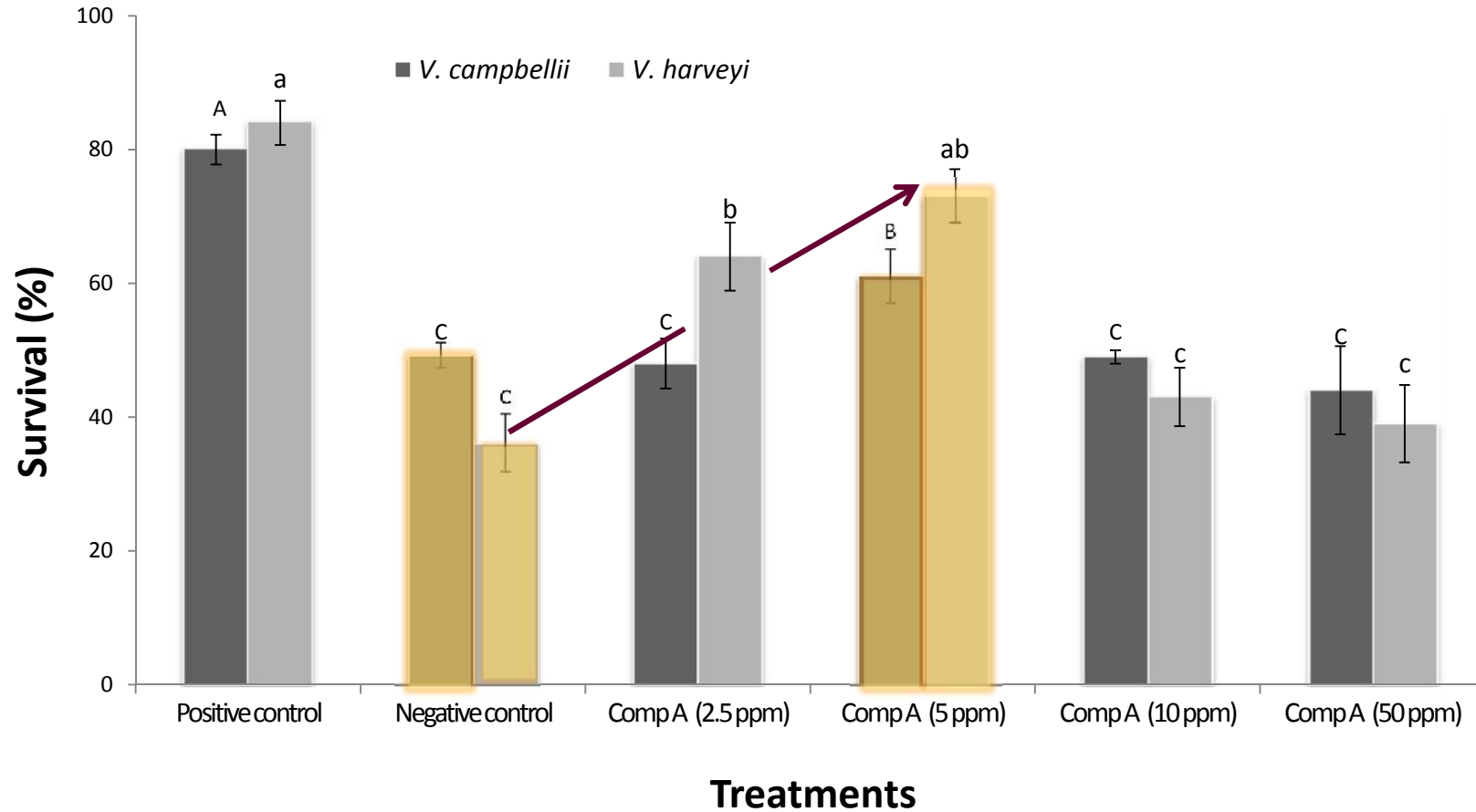


Survival scored after 48 h

RESULTS

Compound A

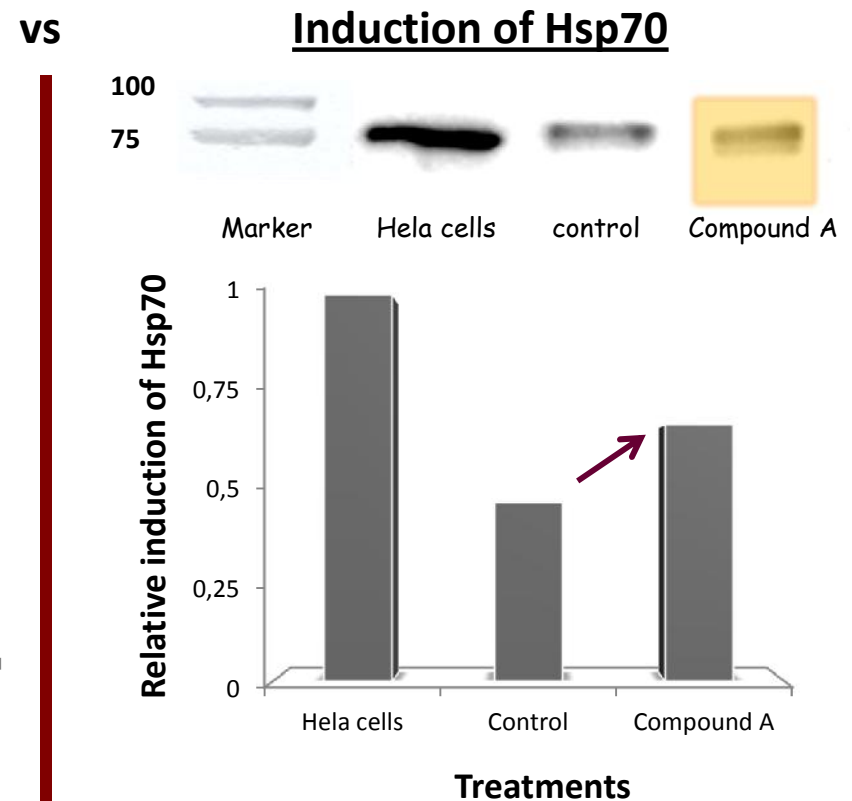
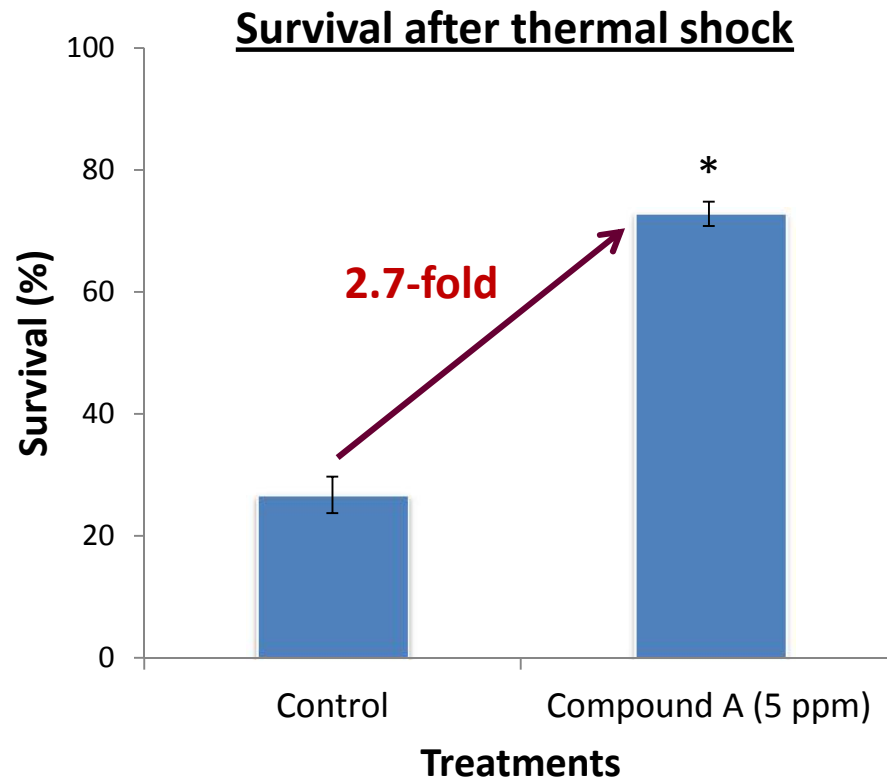
Protective effect of compound A against vibrios



Positive control: No pretreatment, no challenge.

Negative control: No pretreatment, challenged with *Vibrio*

Protective effect of compound A against lethal heat shock



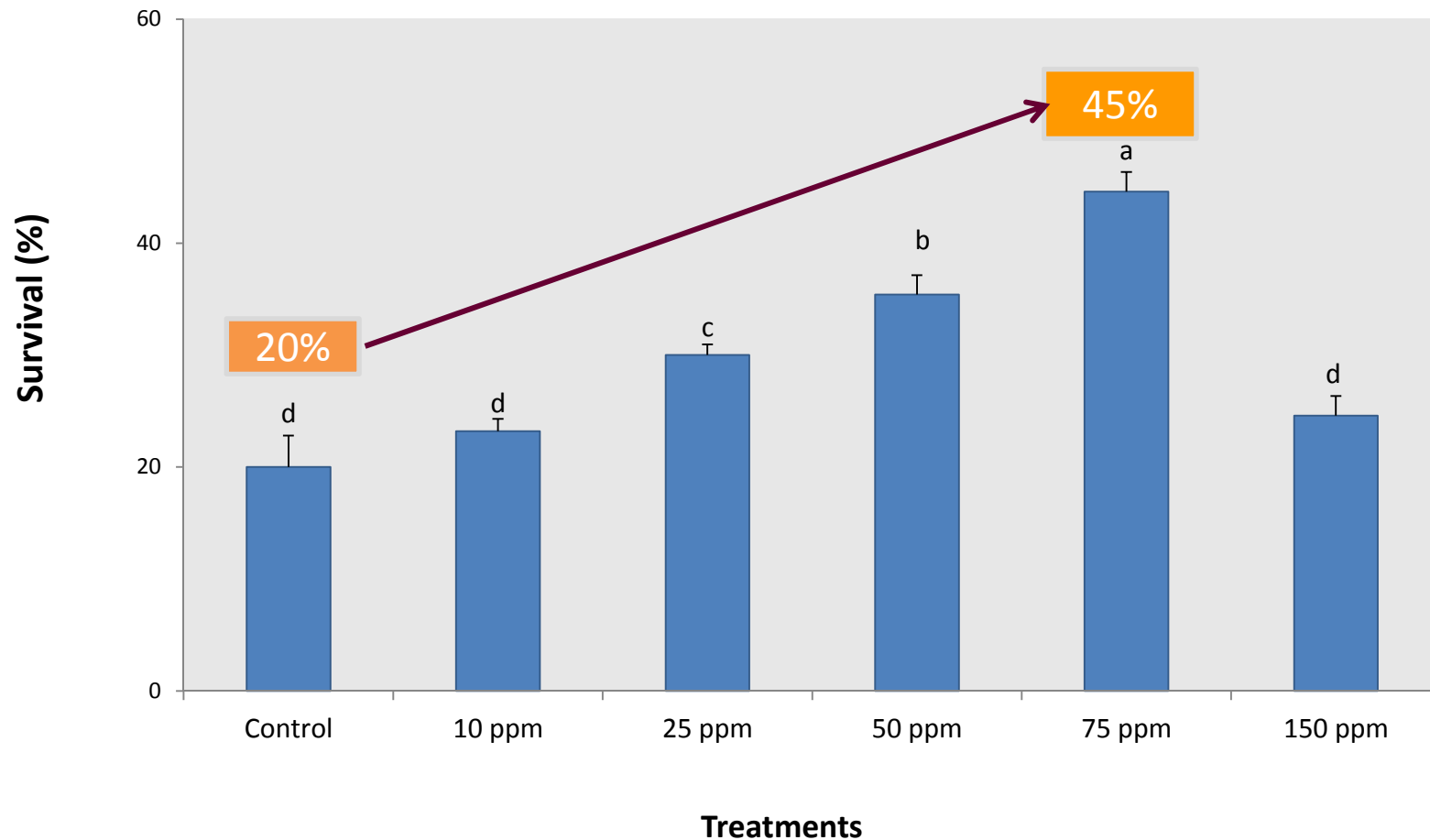
- Compound A conferred protection against vibrios and thermal stress coincides with Hsp70 production

Control: No pretreatment

RESULTS

Compound B

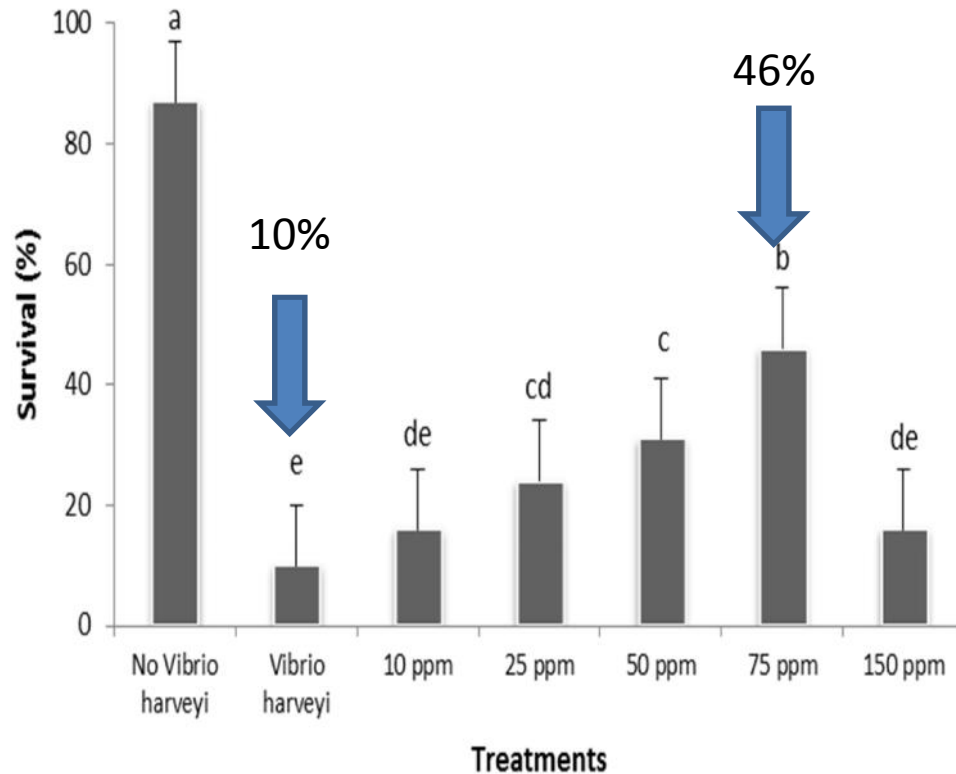
Protective effect of compound B against lethal heat shock



Control: No pretreatment

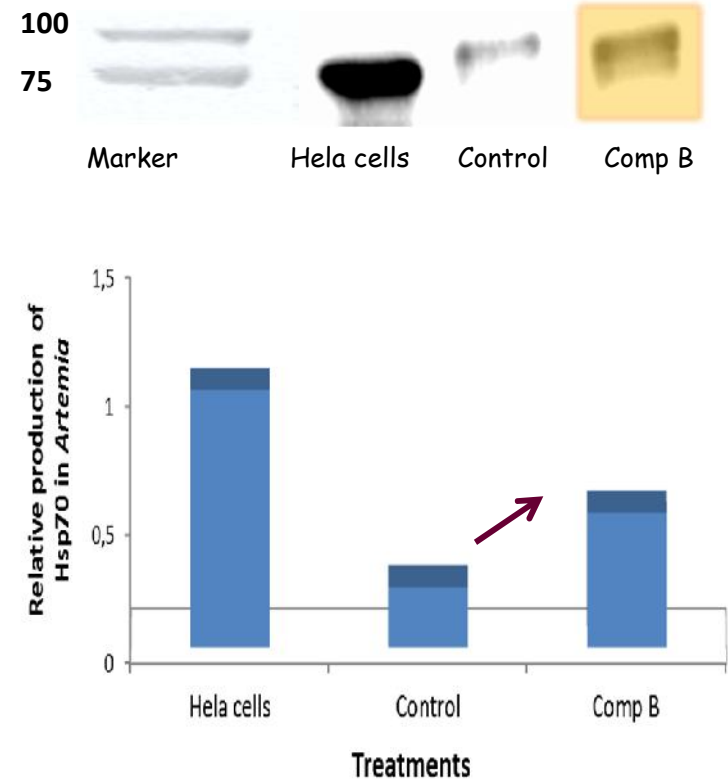
Protective effect of compound B against *V. harveyi*

Survival after *Vibrio* challenge



vs

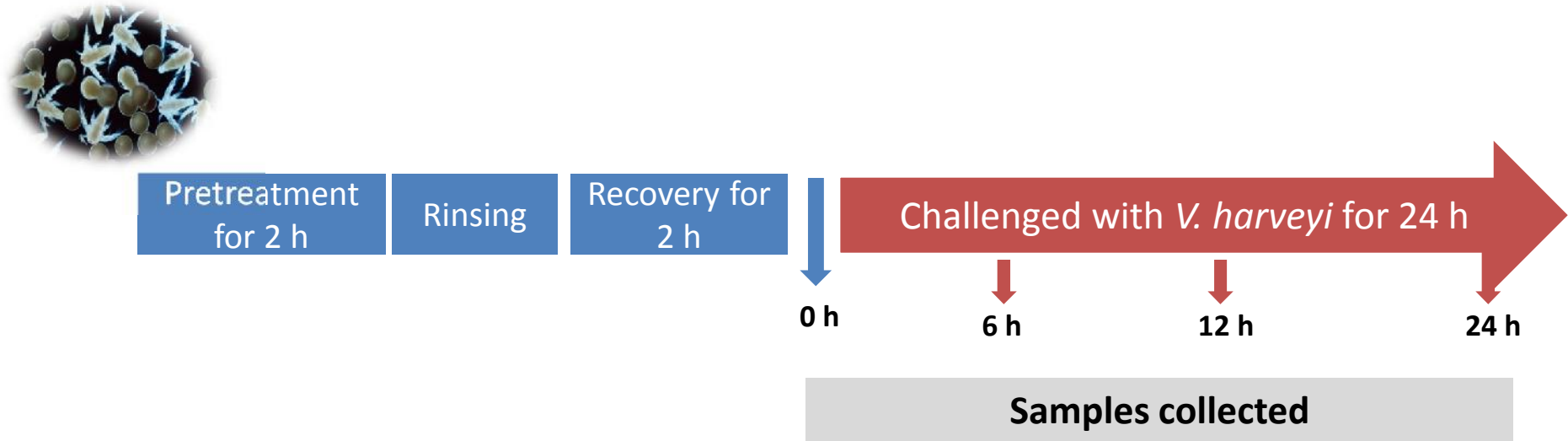
Induction of Hsp70



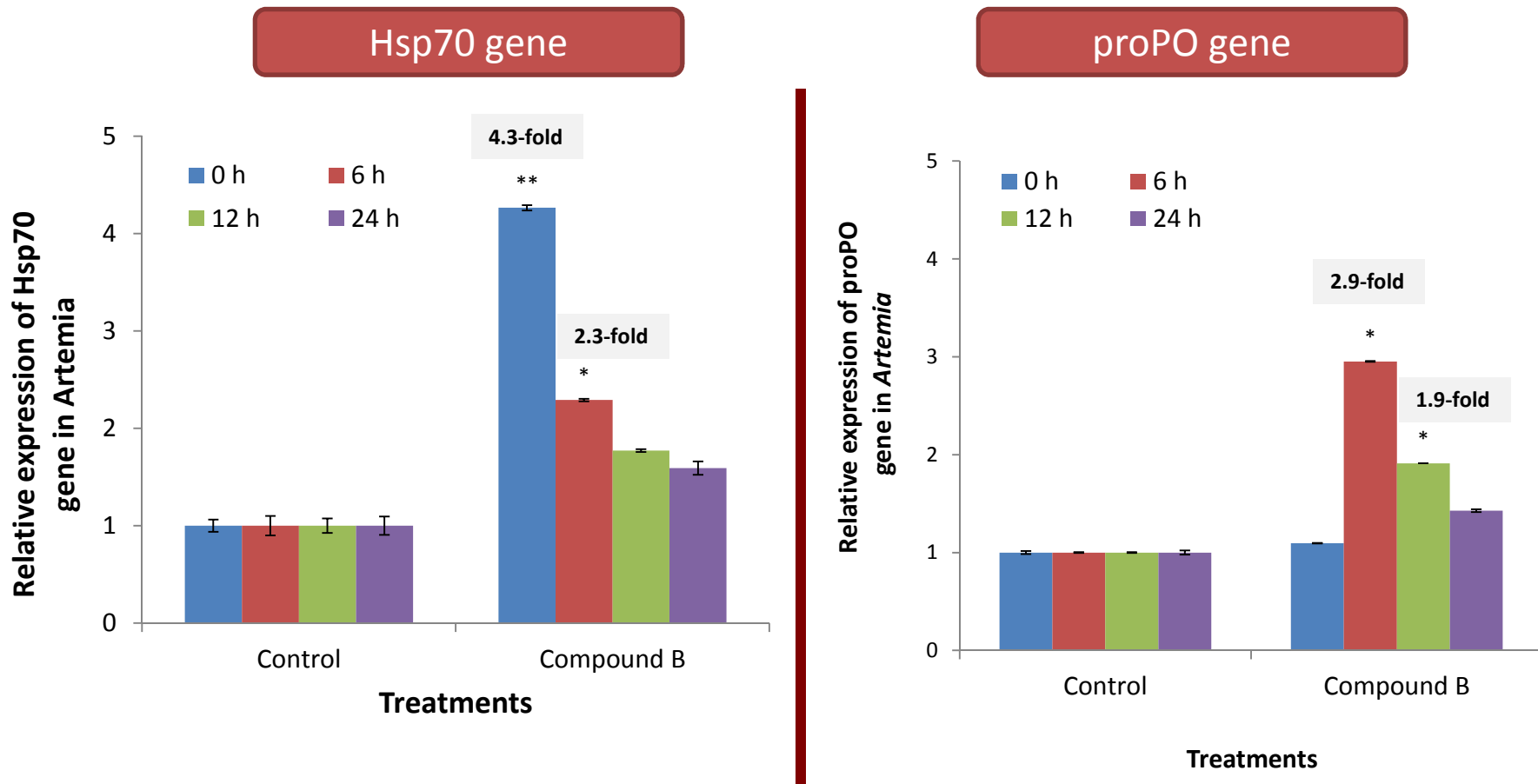
Control: No pretreatment, challenged with *Vibrio*

Experimental design for gene expression by RT-PCR

Collection of samples for Hsp70 and prophenoloxidase (proPO) genes



Effect of Compound B on Hsp70 and proPO genes



Control: No pretreatment

0 h - Pretreatment without *Vibrio* challenge

6, 12 & 24 h: Pretreatment followed by 6, 12 & 24 h of *Vibrio* challenge

Artemia genome

Artemia franciscana

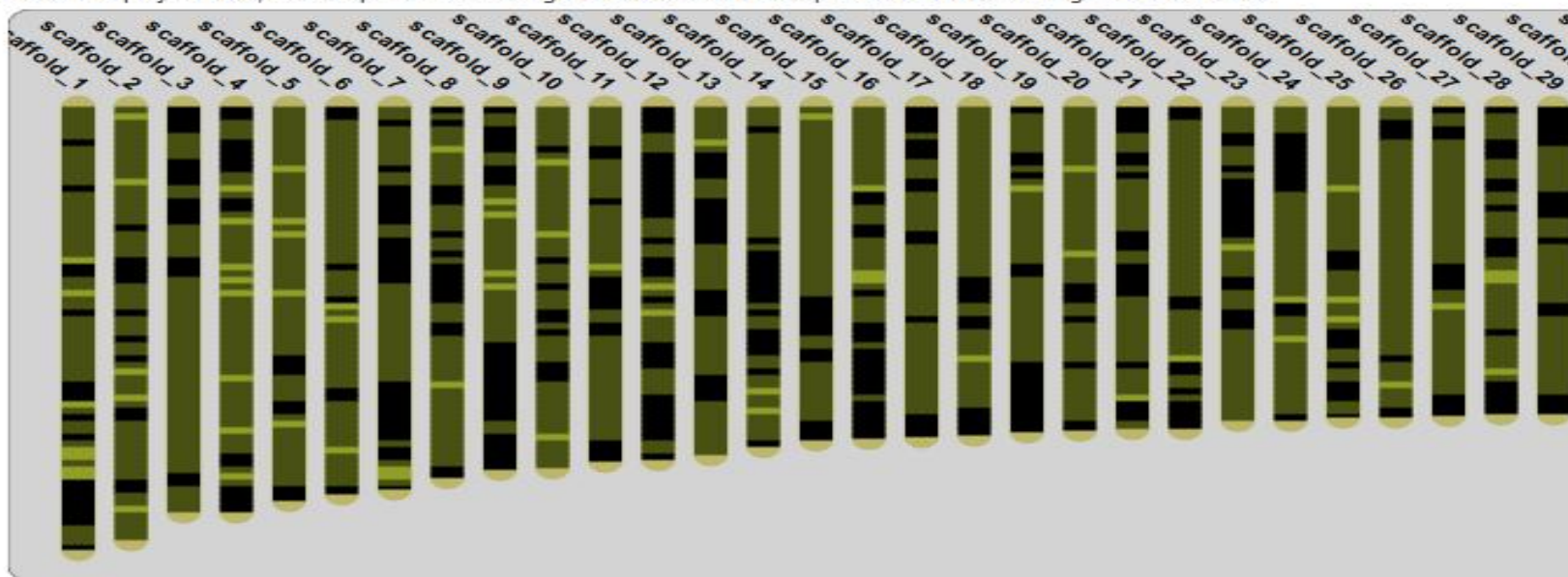


Navigation

▢ BLAST ▢ SEARCH ▢ WIKI ▢ DOWNLOAD ▢ WORKBENCH ▢ WATCHLIST ▢ HELP

Browse

The brighter the color, the higher the gene-density in that region. Click on a region to go to that location in the browser. Only contigs larger than 10Kb are displayed here, the complete list of contigs is available in the dropdown menu from the genome browser.



Annotation

▢ Direct To Gene: **Go!**



CONCLUSIONS

- These plant-extracted compounds are **potential inducers of Hsp70**.
- They **induced tolerance** against subsequent abiotic (thermal) stress.
- They generated **protective immunity** in *Artemia* against pathogenic biotic (vibriosis) stressor by priming the proPO system.
- These compounds represent **potential preventive modality** for vibriosis in *Artemia* and possibly in other commercially important aquaculture species.

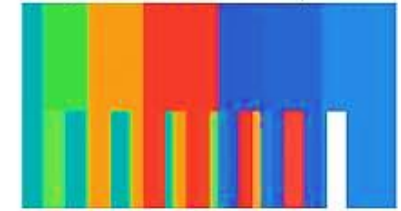


BOF-Special Research Fund



Fonds Wetenschappelijk Onderzoek
Research Foundation - Flanders

Belgian Science Policy Office



belspo

Thank you



Title: Inducer of Heat Shock Protein 70: A New Disease Preventive Option in Aquaculture Production Systems

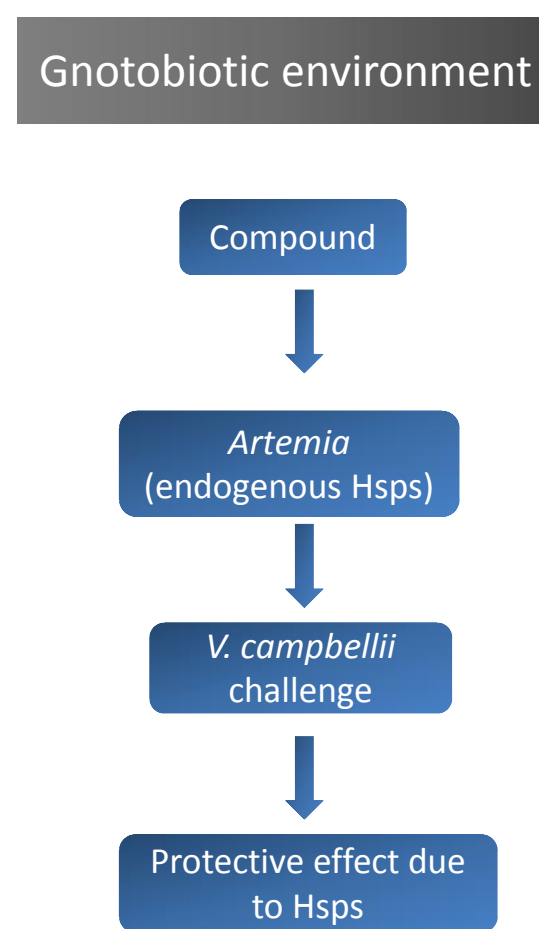
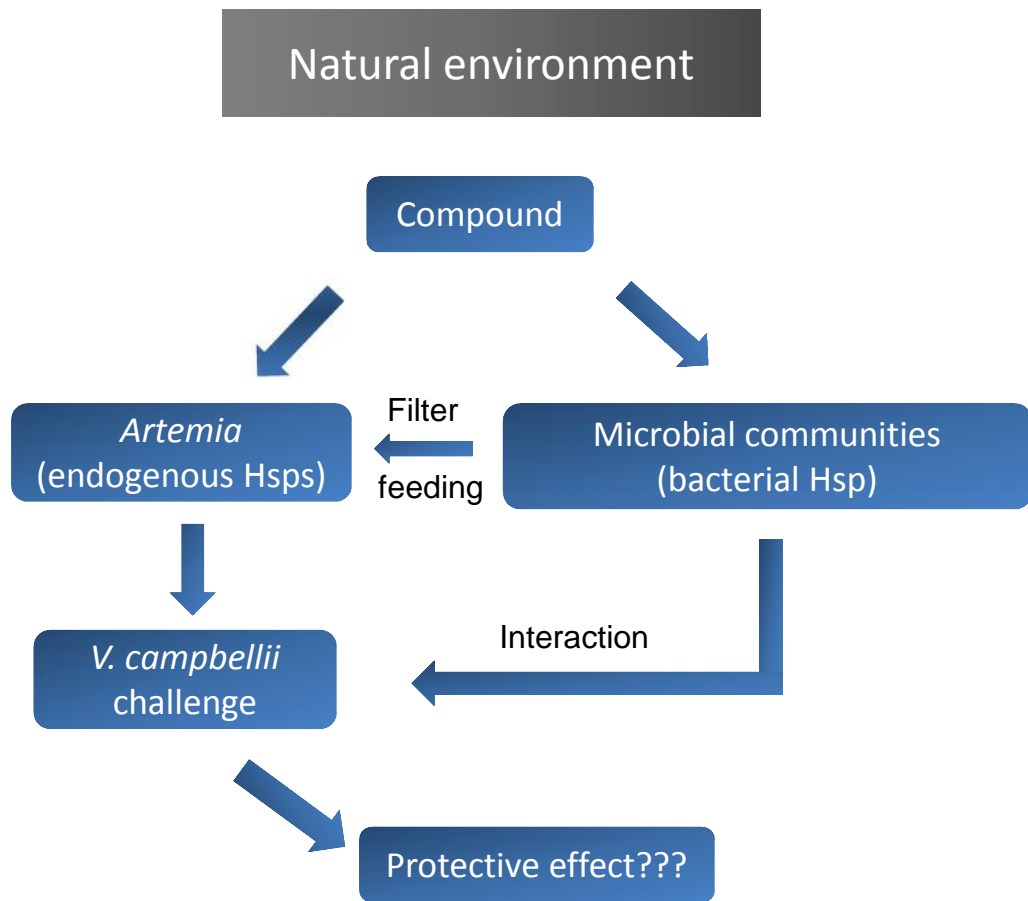
Lab of Aquaculture & *Artemia* Reference Center

27 of 27

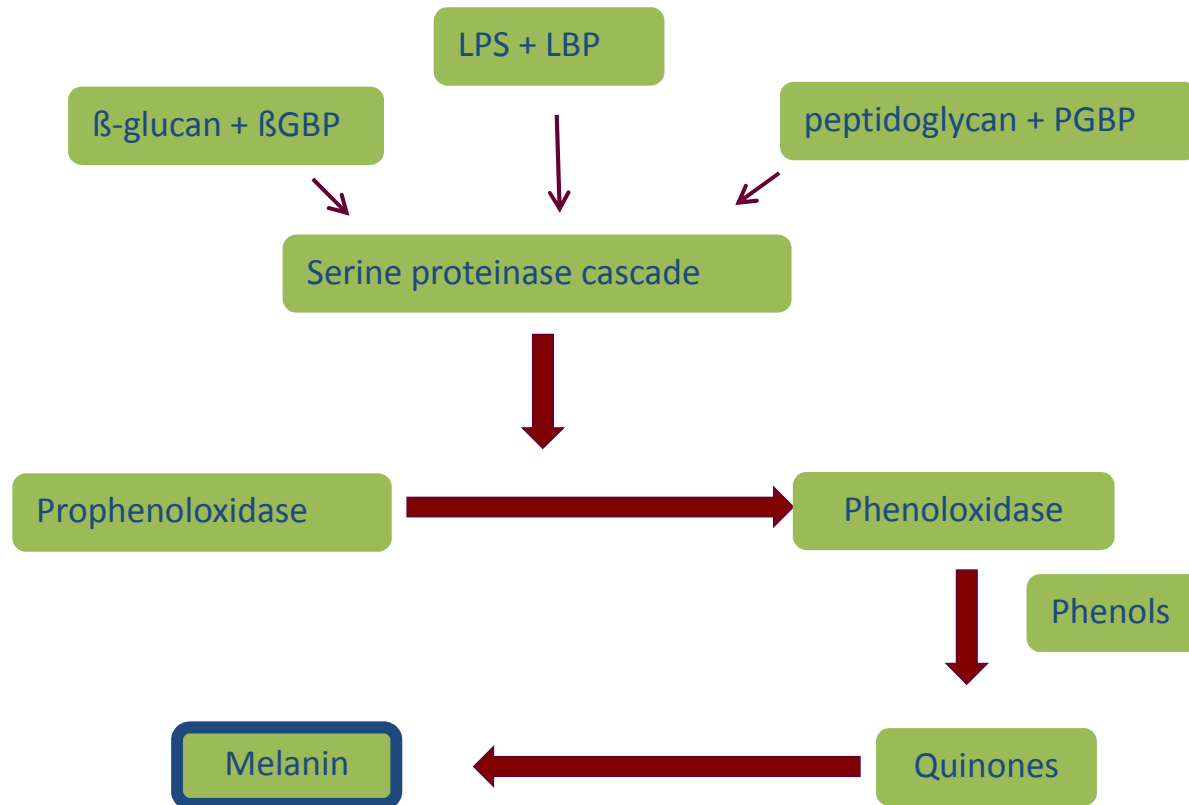


CLARIFICATIONS





Prophenoloxidase Activation system



Experimental Model System

