Recent Developments in Global Shrimp Health Management

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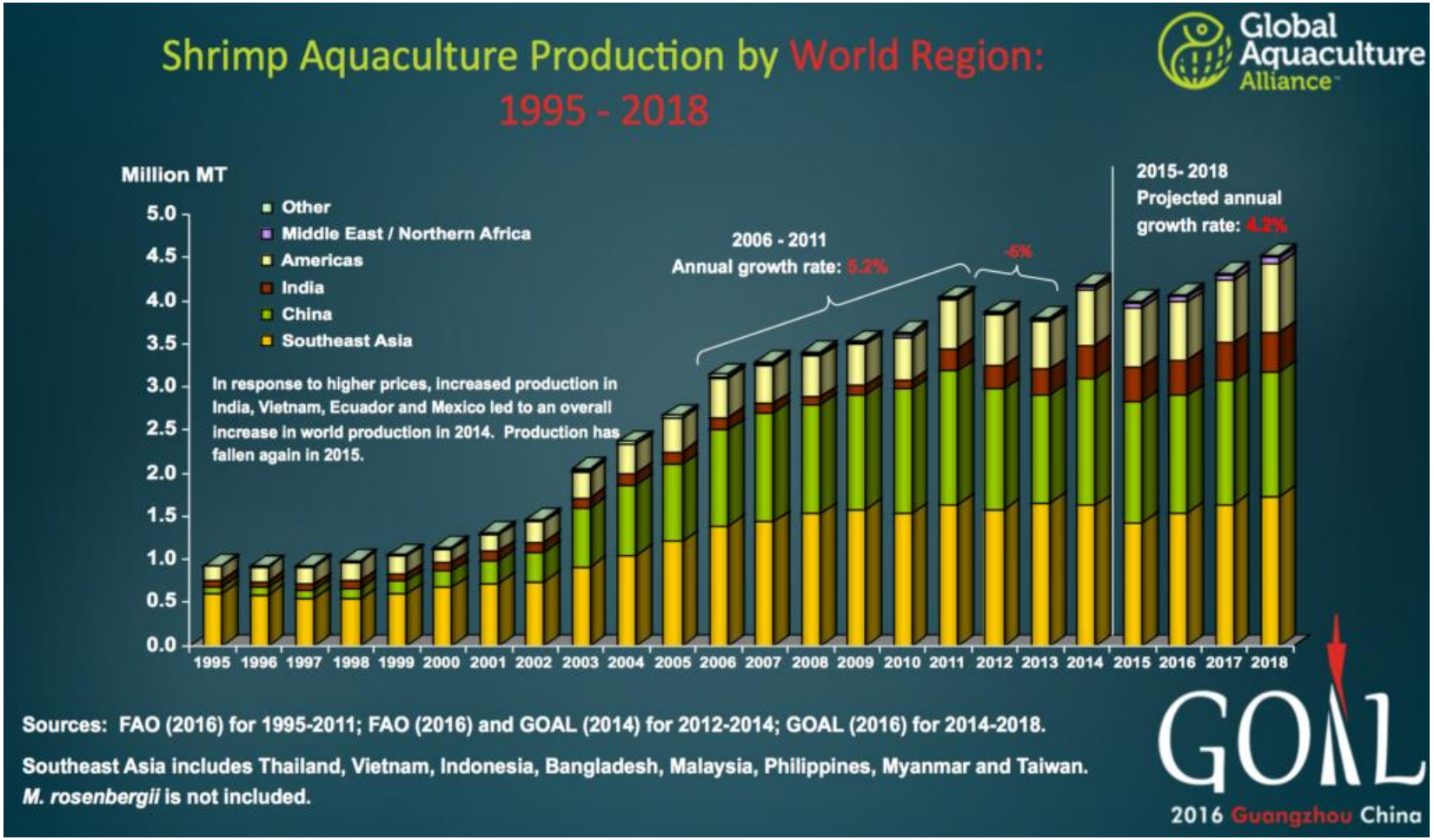
FORUM: PRODUCTION OF PENAID SHRIMP IN EUROPE - RISKS AND OPPORTUNITIES

Shrimp Aquaculture Production

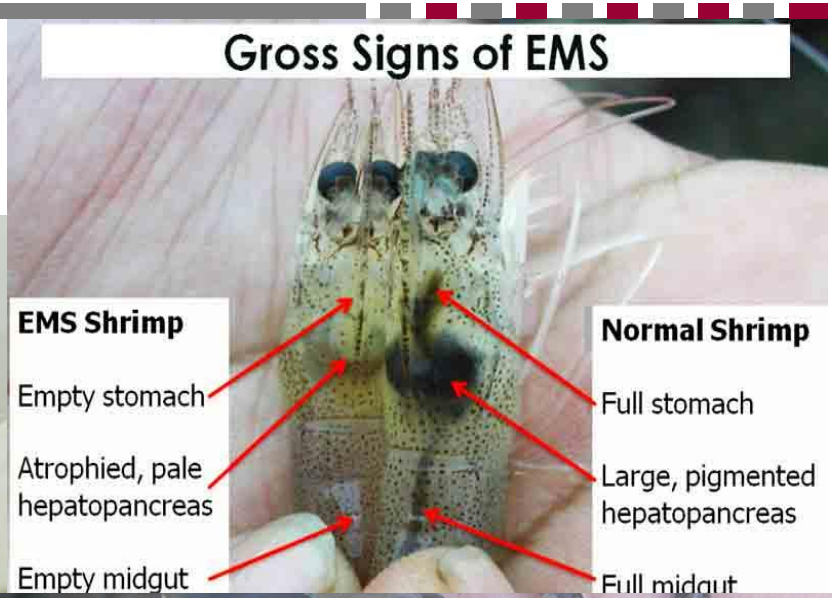


- **The expansion of farmed shrimps is not without diseases**

Early Mortality Syndrome in Shrimps – The Perfect Killer

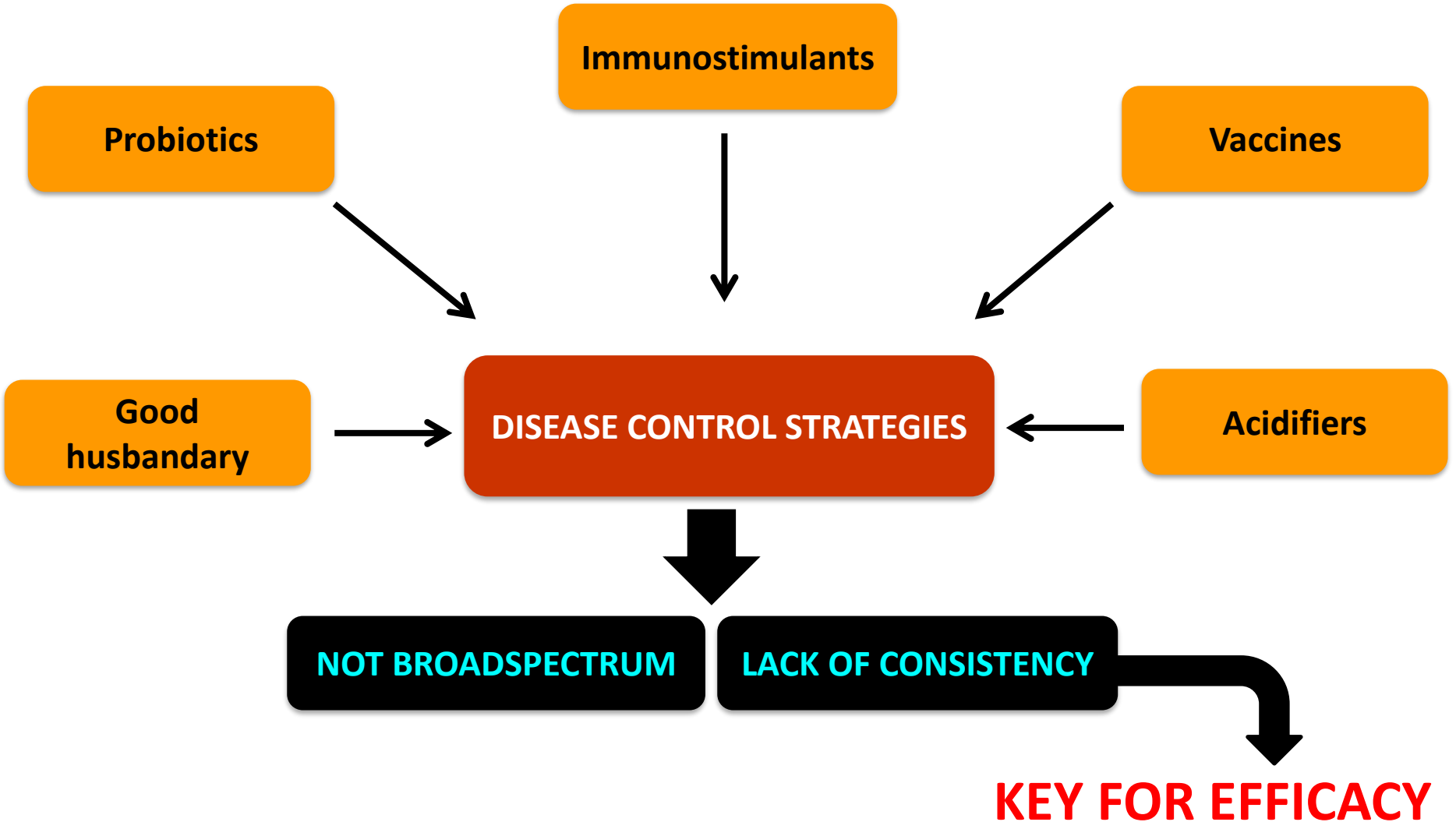
IMPACT: > billion dollar loss

Countries affected: South America, China, Vietnam, Thailand



Juvenile *Penaeus monodon* from Vietnam. Left shrimp appears normal (except for black gills), while right 2 shows dark, atrophied HPs typical of EMS.

Control Measures for Bacterial Diseases in Aquaculture Animals



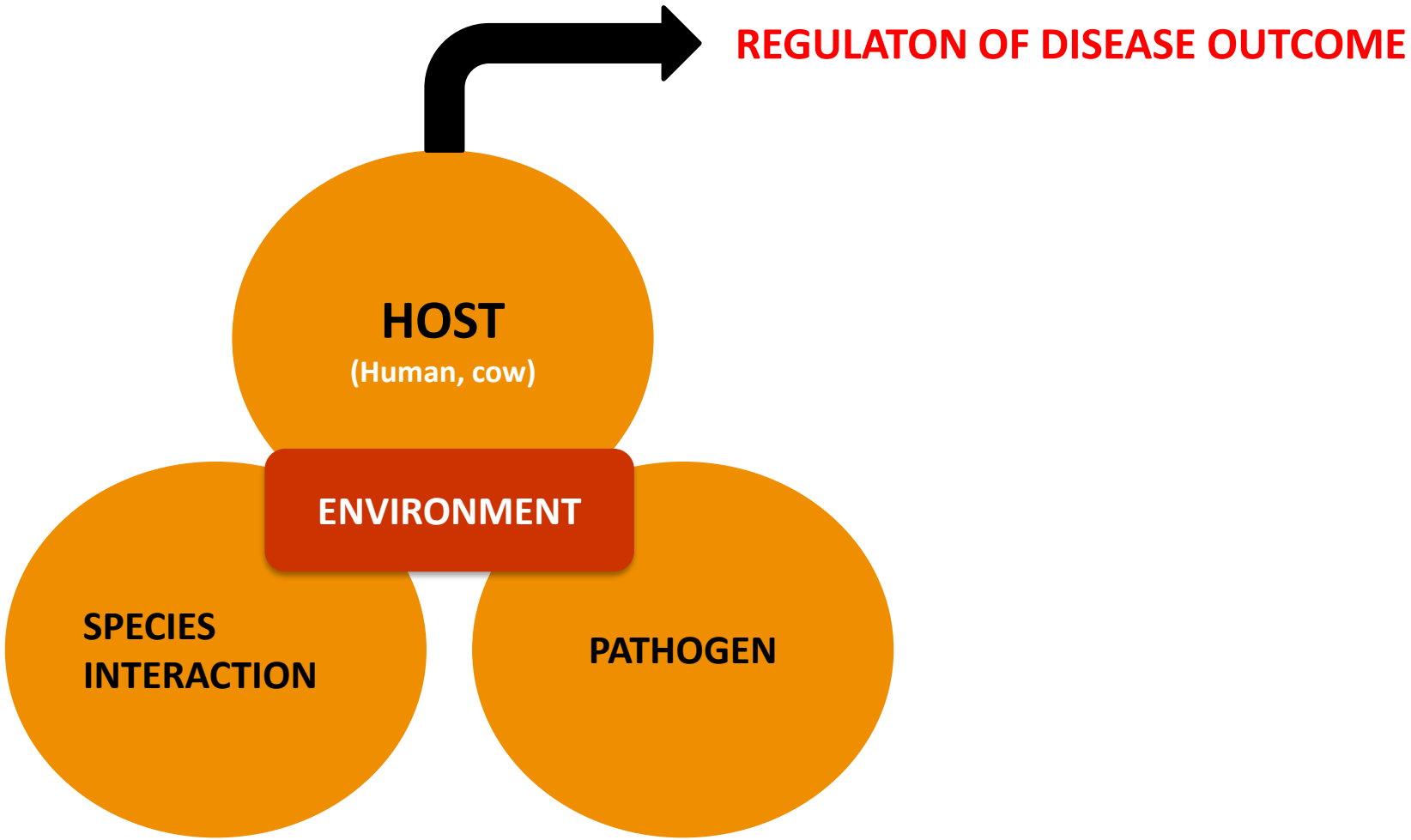
Controlling Diseases in Human – Practice Followed

ISOLATION &
INTENSIVE CARE

Controlling Diseases in Terrestrial Animals – Practice Followed

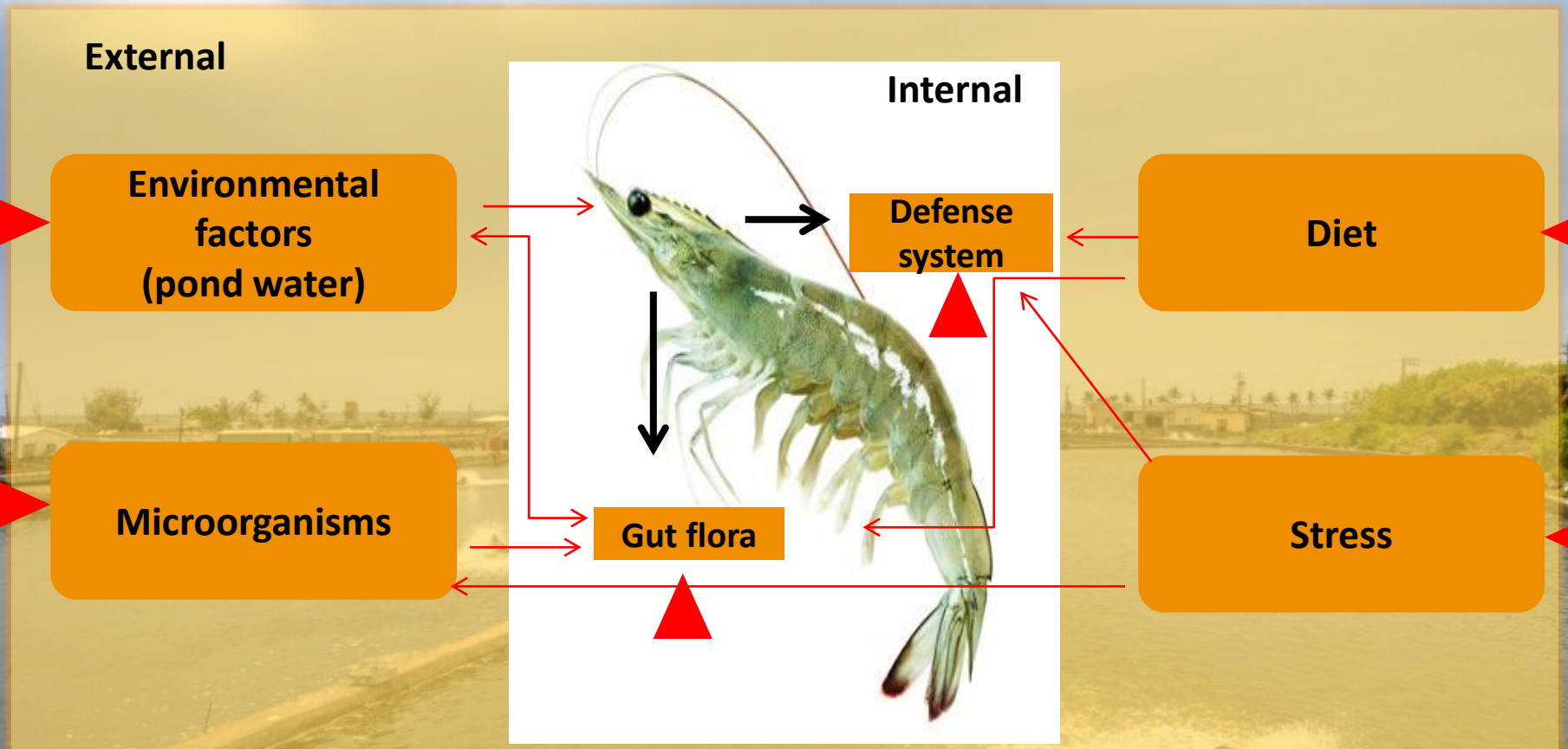
ISOLATION &
INTENSIVE CARE

Role of Environment in Host-Pathogen Interaction

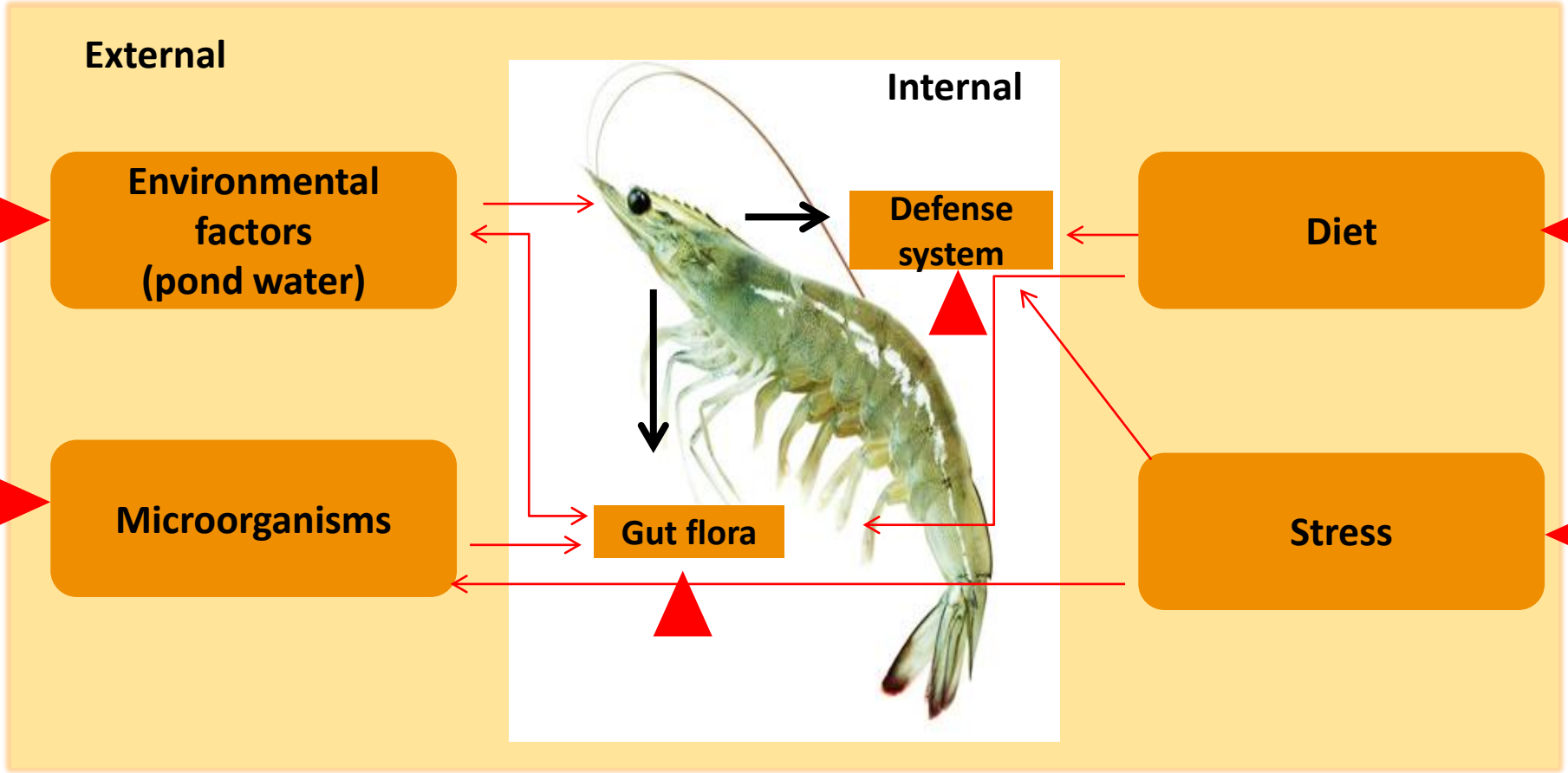


The 3-Dimensional Aquaculture Environment

Multiple agents interact to cause diseases in aqaculture animals



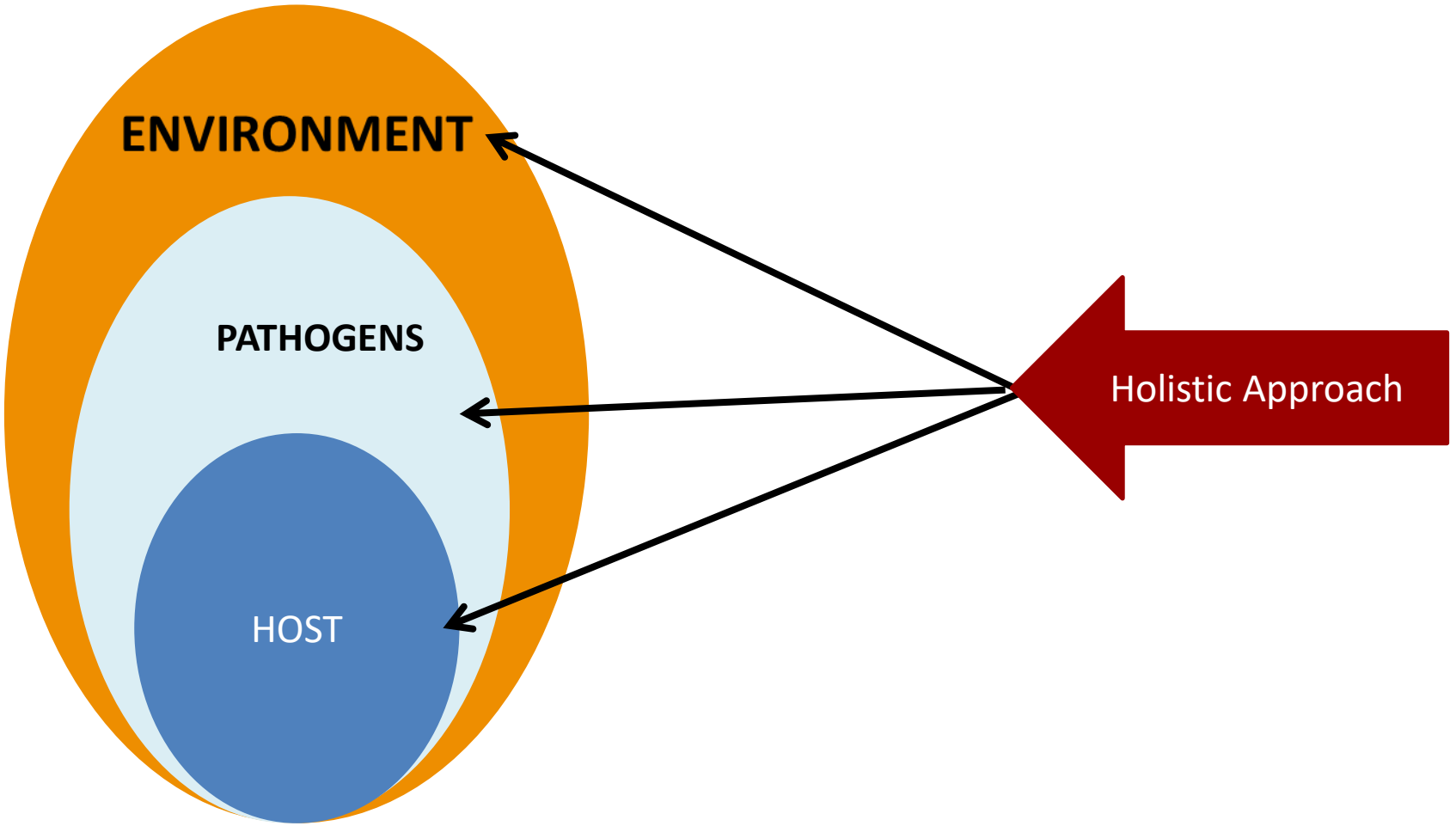
Multiple Agents Cause Diseases in Aquaculture Animals



- Inconsistency of anti-infective strategy – Single disease target

A Paradigm Shift in Shrimp Health Research

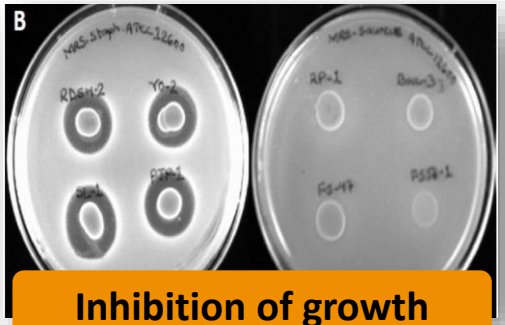
Designing of Anti-infective Strategy



Critical Factor in Host-Pathogen Interaction Studies

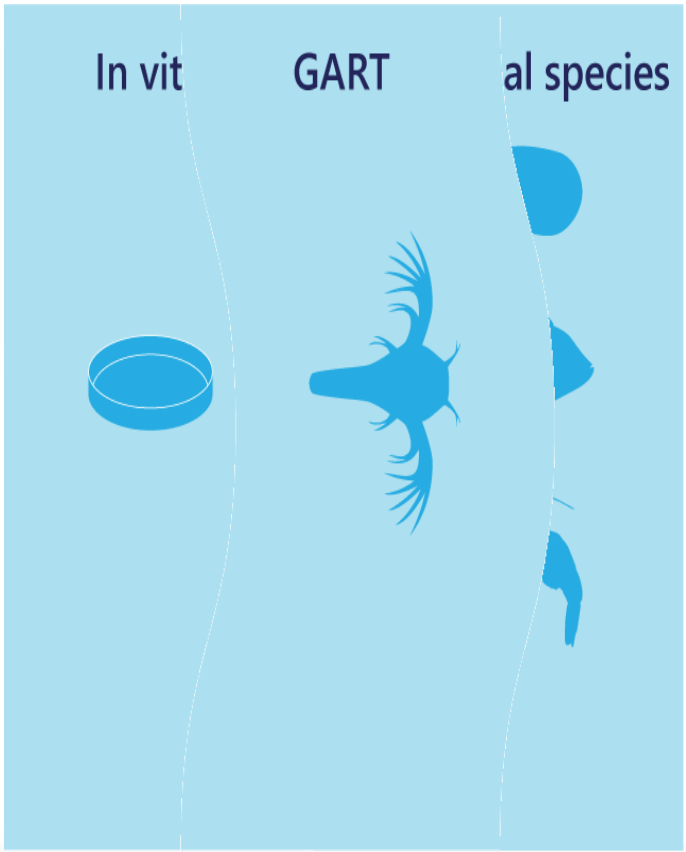
Model organism

Test System - Development of Disease Control Strategies



Inhibition of growth

Virulence factor activity

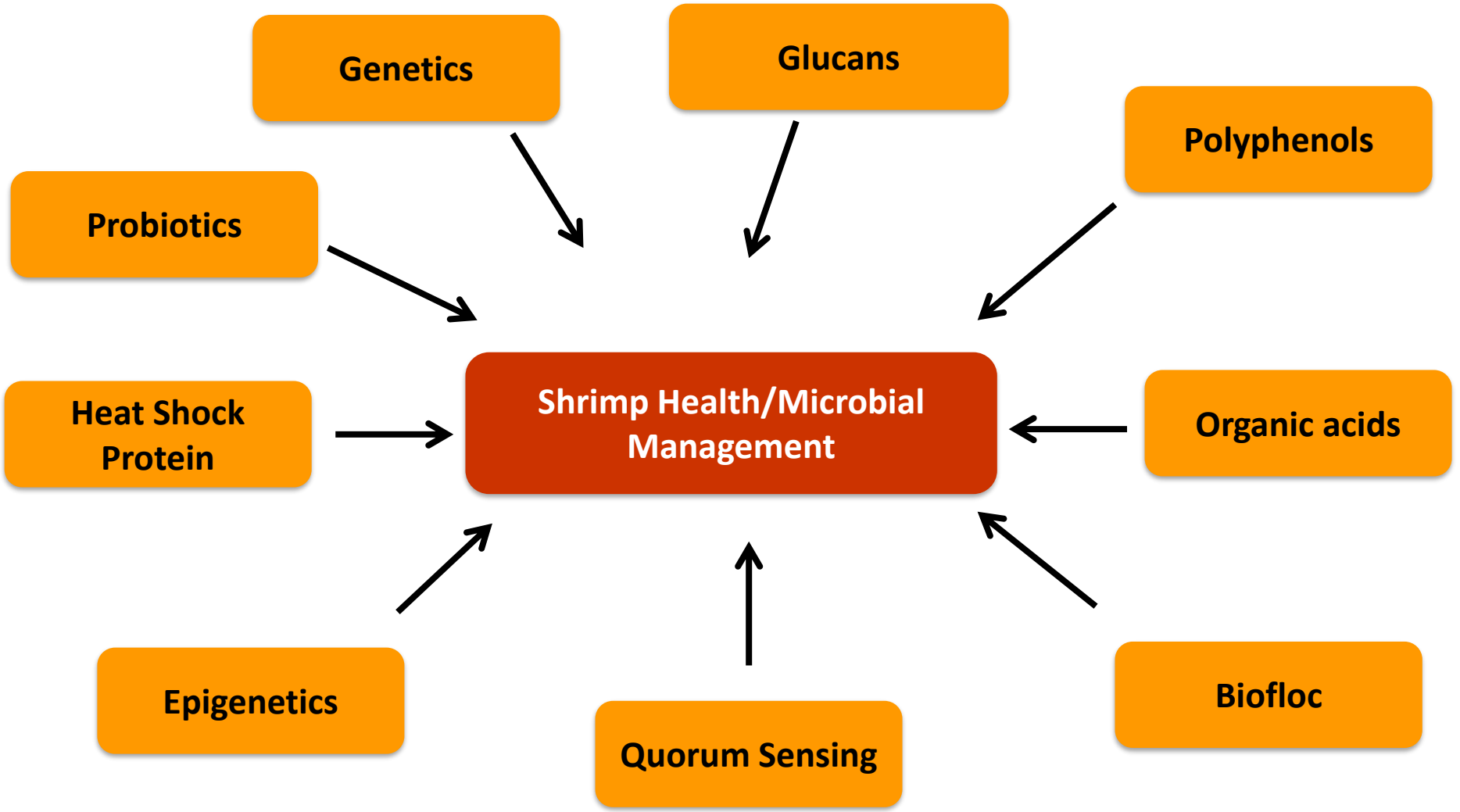


Intermediate step:
GART, the Gnotobiotic *Artemia* screening platform

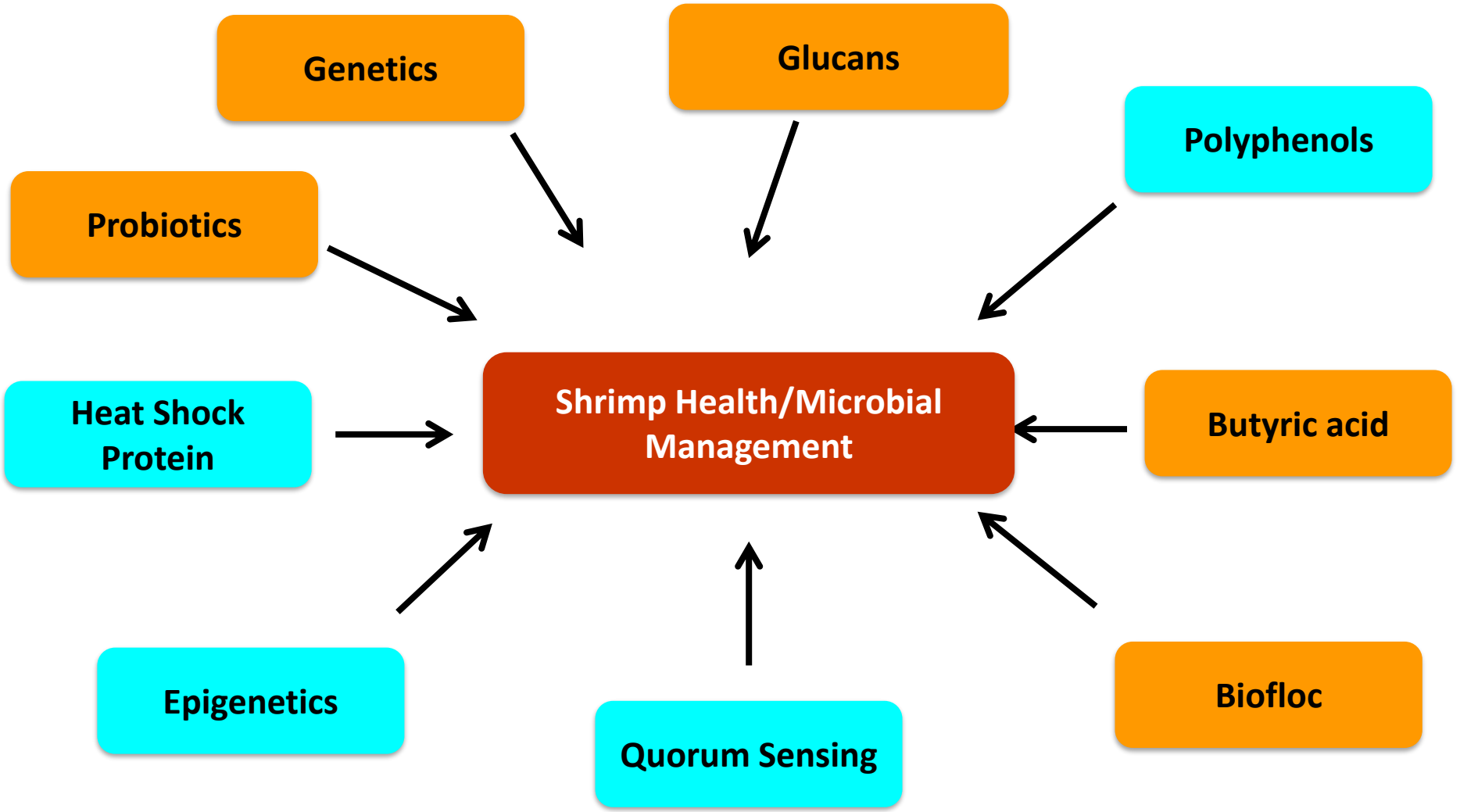
Under real production conditions: trial and error, uncontrolled, risk for production

Under laboratory conditions: no risk for production, more control, yet labor intensive

Shrimp Health and Microbial Management



Findings Snapshot

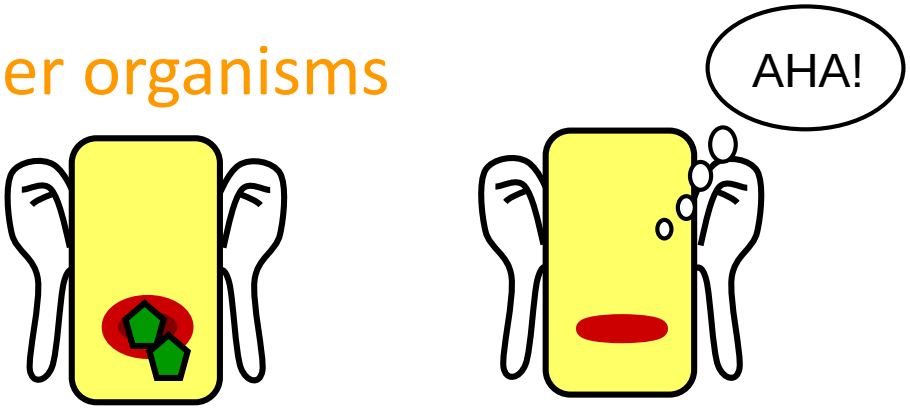


Quorum Sensing

Quorum Sensing

- Before: bacteria = Separate entities

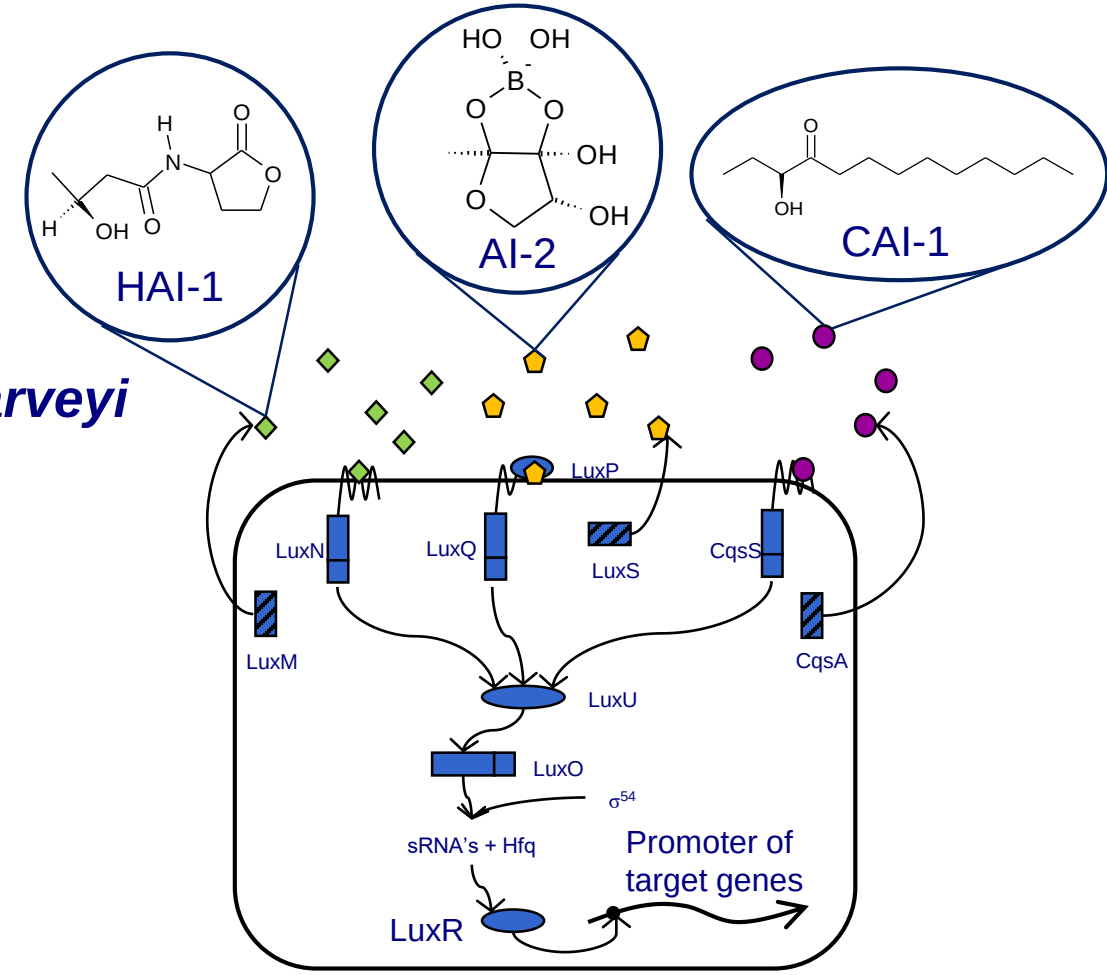
- Now: bacteria sense and respond to environment **and to each other**
  - Extracellular signal molecules
  - ≈ hormones in higher organisms



QUORUM SENSING SYSTEMS

- QS in vibrios: multi-channel systems:

- Documented in:
  - *V. alginolyticus*
  - *V. anguillarum*
  - ***V. campbellii / V. harveyi***
  - *V. ichthyoenteri*
  - *V. mimicus*
  - *V. parahaemolyticus*
  - *V. salmonicida*
  - *V. scophthalmi*
  - *V. vulnificus*



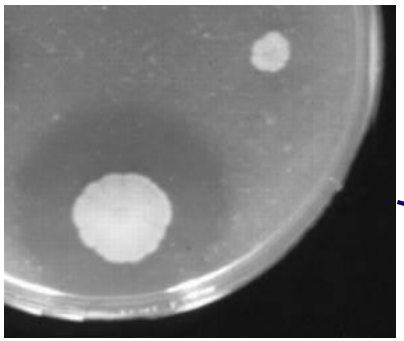
QS-REGULATED PHENOTYPES

- Virulence factors
  - *In vitro* activity assays
  - *In vitro* gene expression (RT realtime PCR)

Quorum sensing

(+) Metalloprotease
"Extracellular toxin"

(-) Type III secretion system
Siderophore
Phospholipase
Chitinase

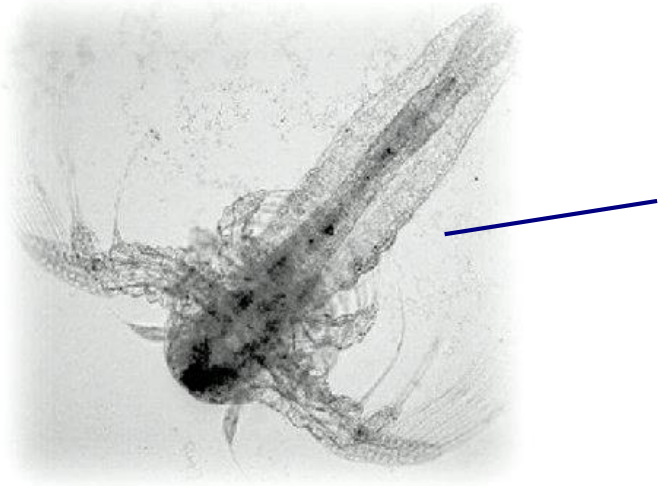


Chitinase assay

More info: Ruwandeepika et al. (2012) Reviews in Aquaculture 4: 59-74

INTERACTION: Vibrio – brine shrimp

- Brine shrimp (*Artemia*): model organism for shrimp

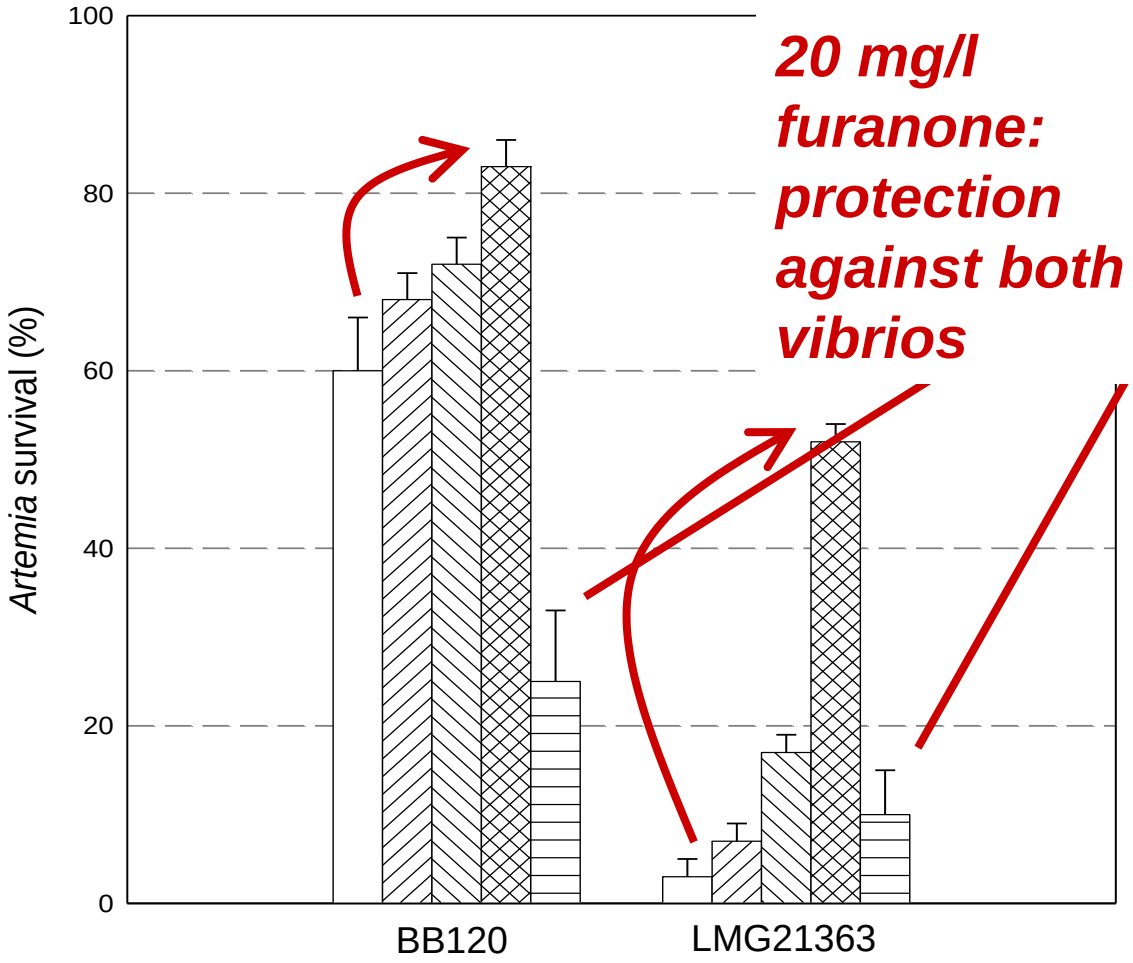


- Creating gnotobiotic cultures starting with axenic larvae (Instar II nauplii)

  → Only bacteria added to cultures are present!

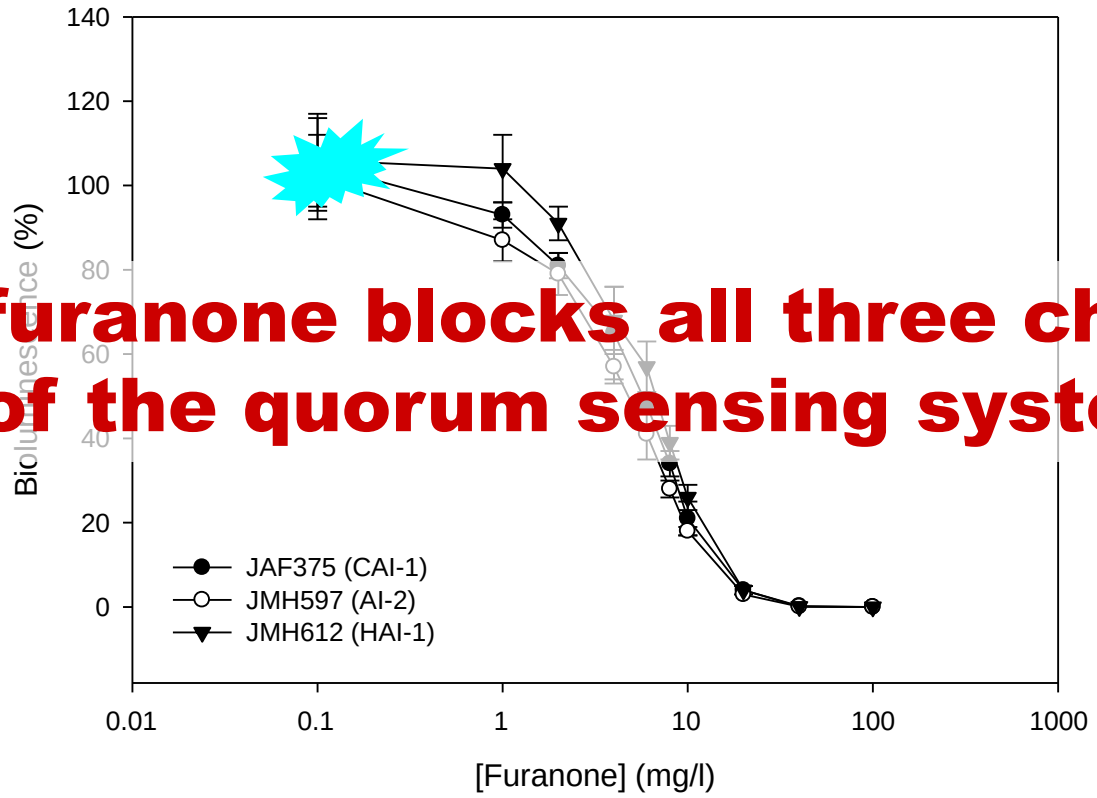
Artemia Model in Bacterial QS Research

- *In vivo* challenge tests with gnotobiotic *Artemia* and weak (BB120) or more virulent (LMG21363) strain



Disruption of QS to Control Bacterial Infections

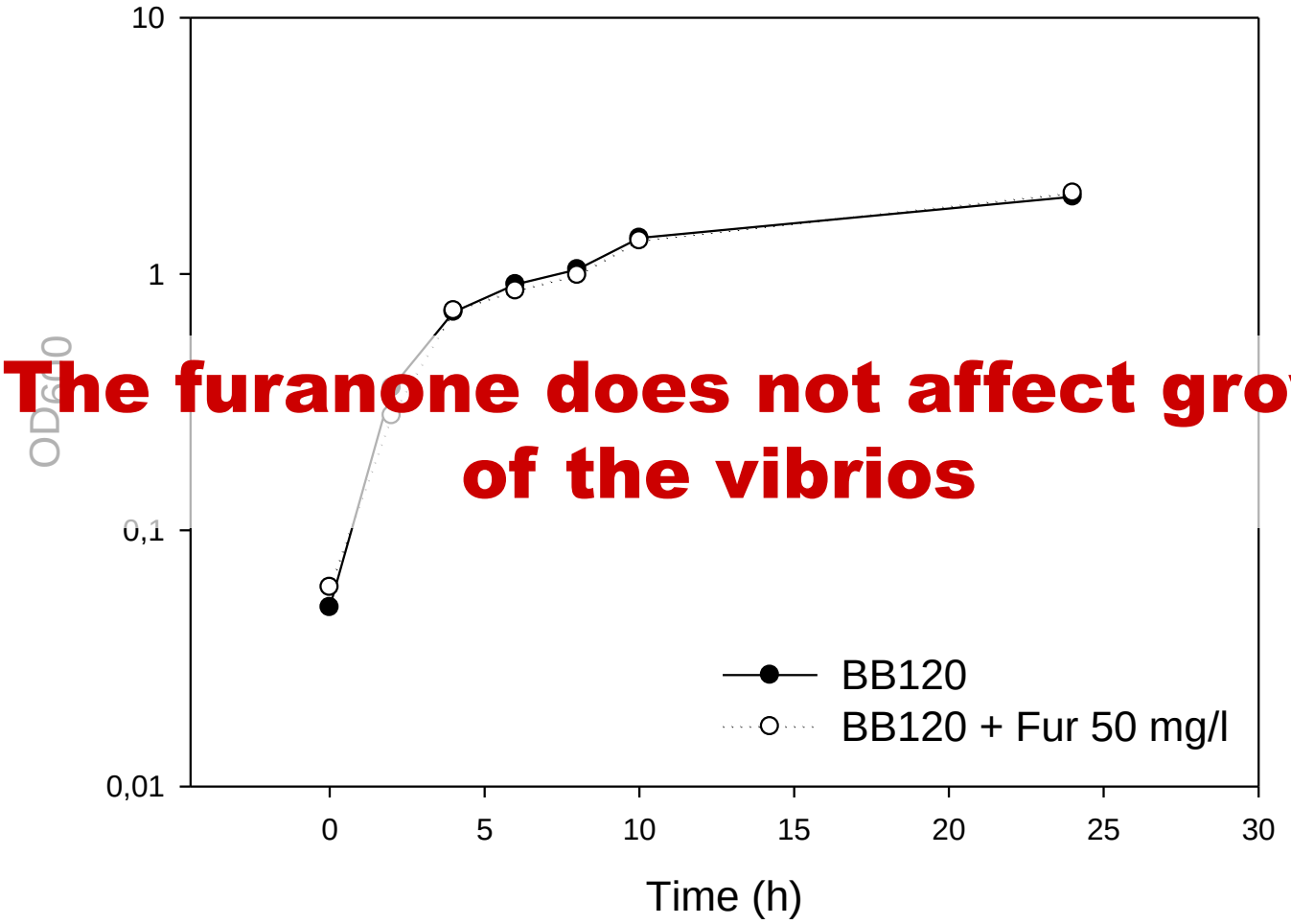
- **Use of QS inhibitors** (e.g. plant extracts/plant-derieved compound)
- Effect of the furanone on bioluminescence of *V. harveyi*



The furanone blocks all three channels of the quorum sensing system

Disruption of QS to Control Bacterial Infections

- *In vitro* growth of luminescent vibrios without and with furanone (50 mg/l)



The furanone does not affect growth of the vibrios

Epigenetics

Teaching Shrimps Self-Defence to Fight Infections

Trends in Biotechnology

CellPress
REVIEWS

Forum

Teaching Shrimps Self-Defense to Fight Infections

Parisa Norouzitallab,[1,2,4,*]
Kartik Baruah,[2,3,4]
Daisy Vanrompay,[1,5] and
Peter Bossier[2,5]

impose major yield-limiting effects on production, causing significant losses [5]. To avoid production losses, several veterinary drugs are commonly used. However, their imprudent use has resulted in the development of antimicrobial resistance (AMR) in many shrimp pathogens (including bacteria, fungi, viruses, and parasites). Consequently, veterinary drugs are no longer effective in treating shrimp dis-

more predictable, reliable, cost-effective, and ultimately more sustainable disease-control strategy. The results of a trans-generational study using the shrimp model organism *Artemia* suggested that training induced by exposing the parental population of *Artemia* at their early life stages to challenge with *Vibrio campbellii* (an important shrimp bacterial pathogen) significantly increased the resistance of

Experimental Design

Parthenogenetic *Artemia*

(clonal population)

Daily non-lethal heat shocks (T)

Iso-thermic (C)

TF1

TF2

TF3

ISO THERMIC

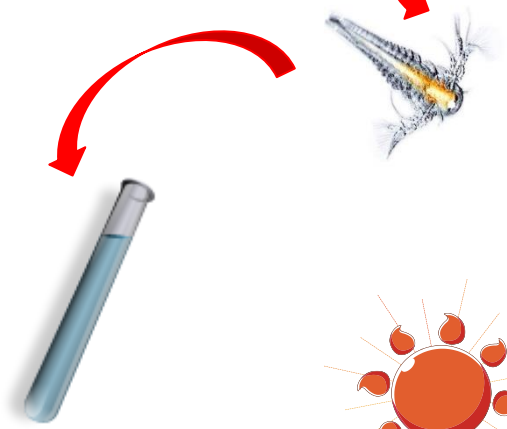
CF1

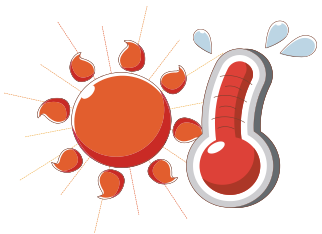
CF2

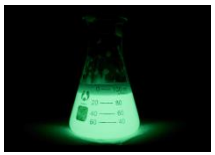
CF3

Common garden test - Verifying stress-resistant phenotypes

F1 F2 F3







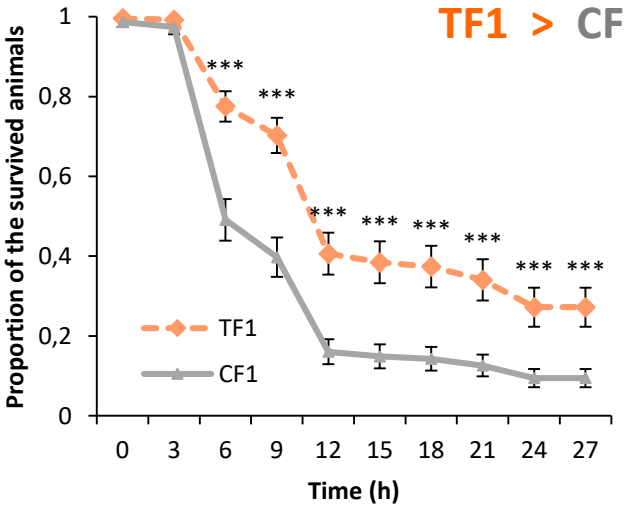
20 instar II nauplii in 6 replicates

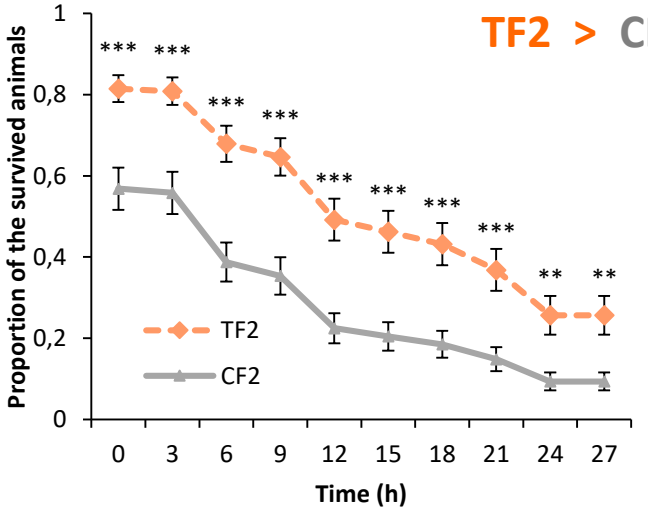
42°C for 15 min

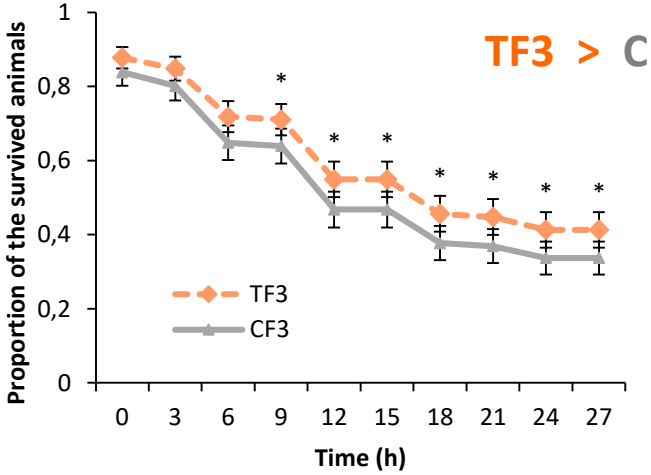
Or

Vibrio campbellii 10^7 cells/ml

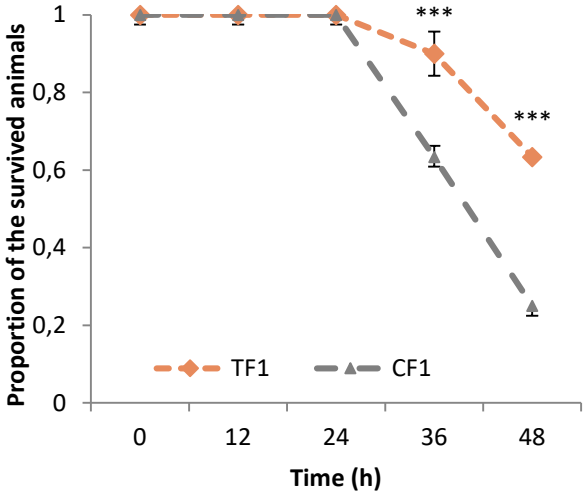
Thermo-tolerance test



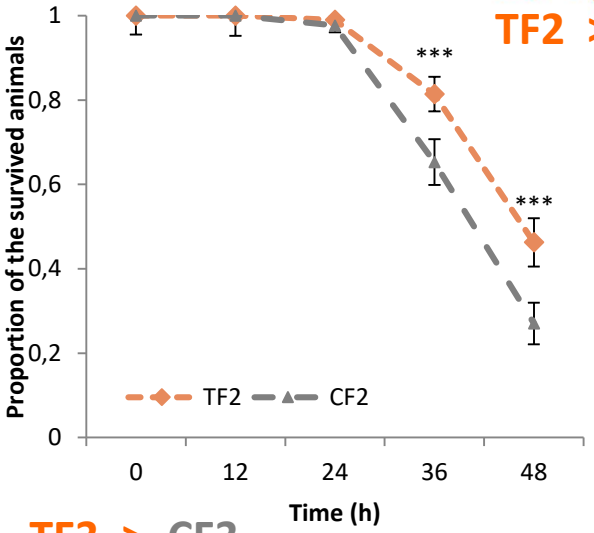




V. campbellii resistance



TF1 > CF1



TF2 > CF2

TF3 > CF3

The FASEB Journal • Research Communication

Environmental heat stress induces epigenetic transgenerational inheritance of robustness in parthenogenetic *Artemia* model

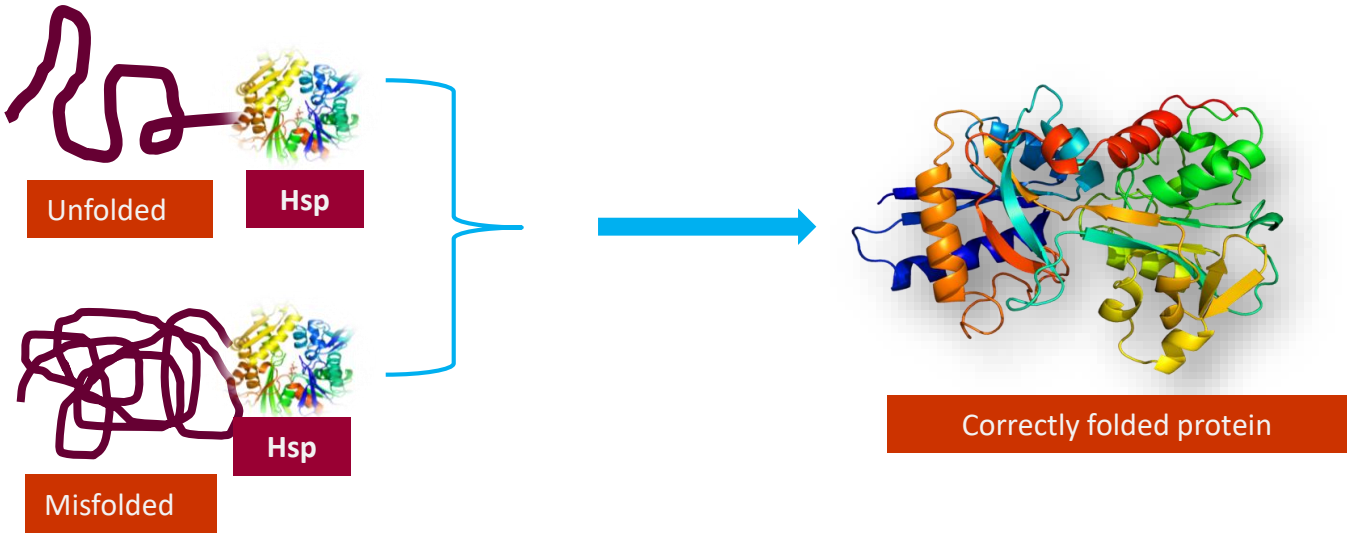
Parisa Norouziallab,[*,†] Kartik Baruah,[*,†] Michiel Vandegehuchte,[‡] Gilbert Van Stappen,[*,†] Francesco Catania,[§] Julie Vanden Bussche,[‖] Lynn Vanhaecke,[‖] Patrick Sorgeloos,[*,†] and Peter Bossier[*,†,1]

[*]Laboratory of Aquaculture, [†]*Artemia* Reference Center, and [‡]Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Ghent, Belgium; [§]Institute for Evolution and Biodiversity, University of Münster, Münster, Germany; and [‖]Laboratory of Chemical Analysis, Ghent University, Merelbeke, Belgium

HSP-INDUCING COMPOUNDS IN HEALTH & DISEASE

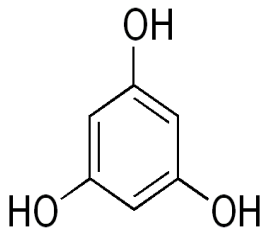
Function of Intracellular Hsp

- Conserved proteins acting as molecular chaperones - bind to unfolded proteins (nascent polypeptides or denatured ones) - facilitate their refolding to the native state

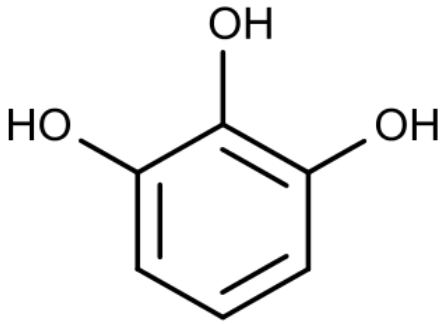


- Involved in protein translocation and degradation
- Immunogenic proteins that modulate both innate and adaptive immune responses

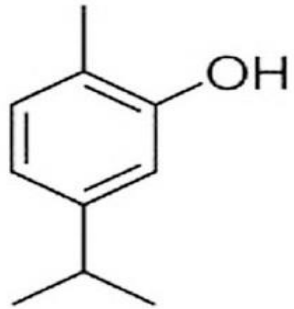
Hsp-inducing Compound in Shrimp Health Management



Phloroglucinol



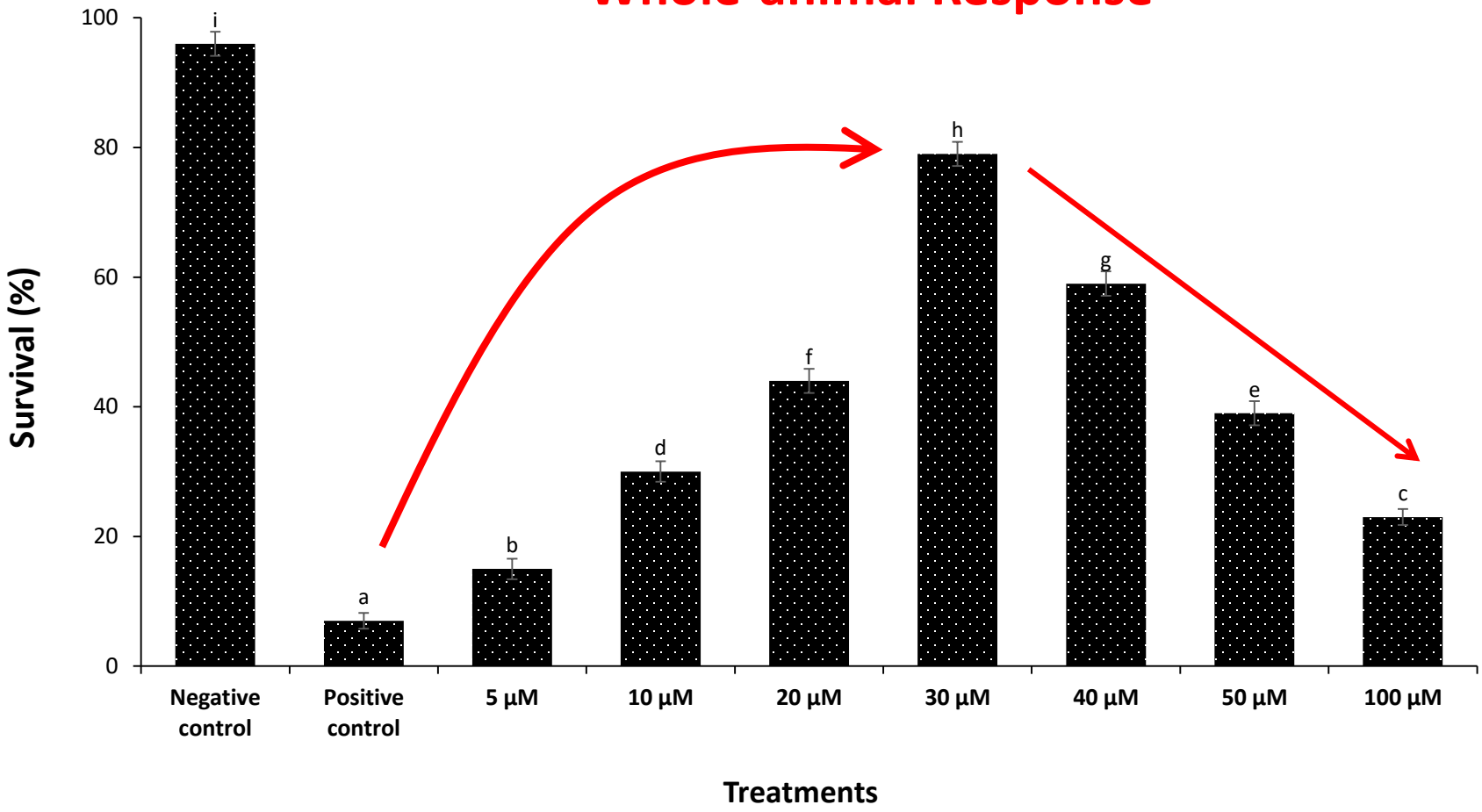
Pyrogallol



Carvacrol

Protective Effect of Phloroglucinol against *V. parahaemolyticus*

"Whole-animal Response"

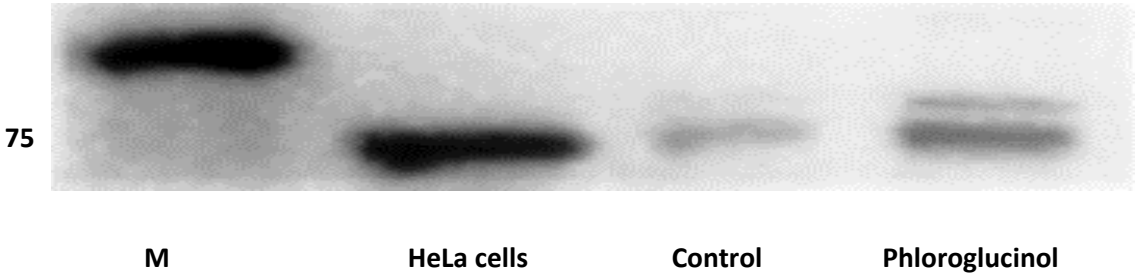


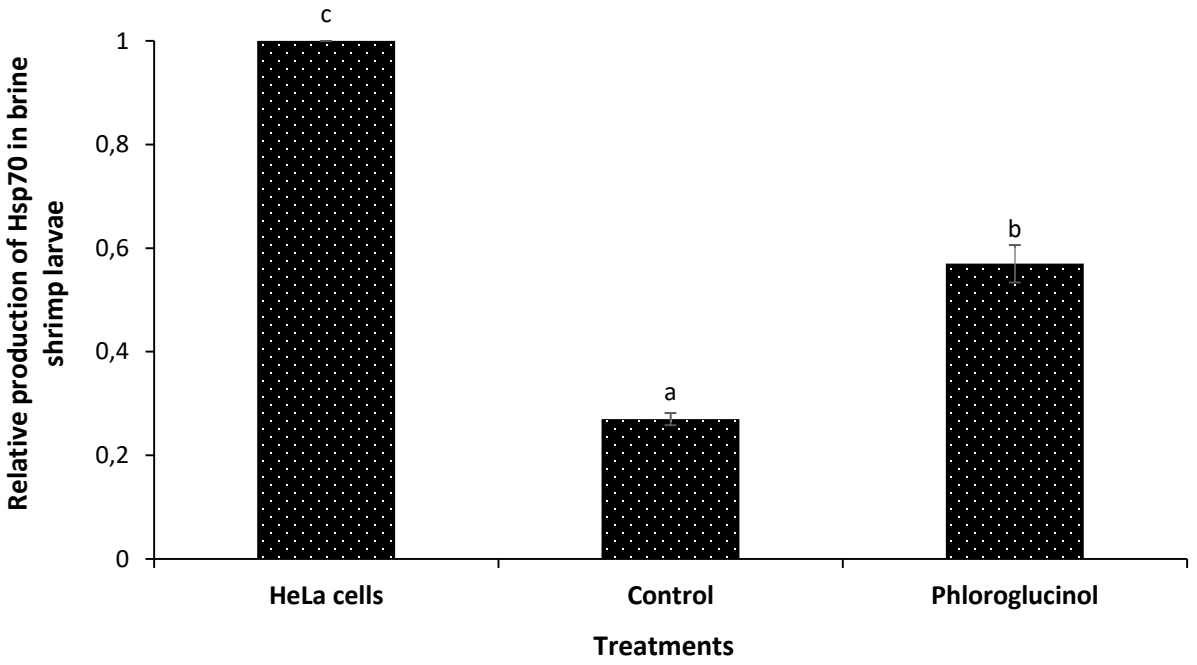
Negative control: No pretreatment, no challenge

Positive control: No pretreatment, *Vibrio* challenge

Kumar et al. 2018. Frontiers in Immunology. 9:1091

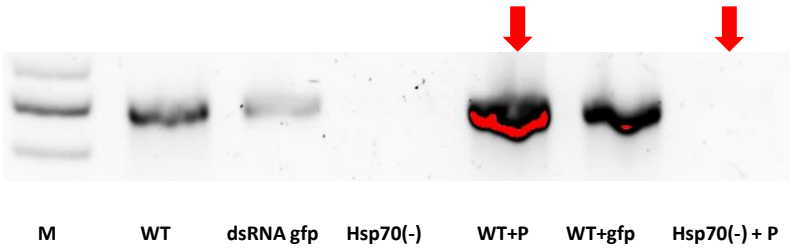
Mode of Action of Phloroglucinol: Induction of Hsp70

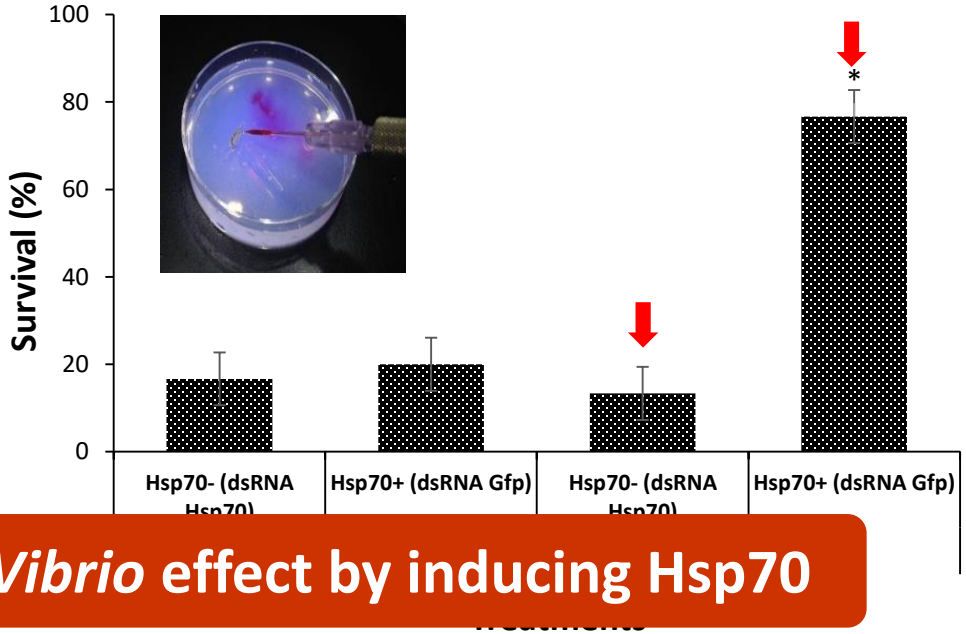




Kumar et al. 2018. Frontiers in Immunology. 9:1091

In vivo knockdown of *Artemia* Hsp70 using RNAi





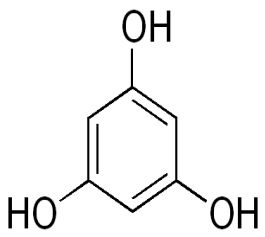
Phloroglucinol induces anti-*Vibrio* effect by inducing Hsp70

Figure: Knockdown of *hsp70* mRNA in *brine shrimp* larvae. Equal amounts of RNA from *brine shrimp* larvae were amplified by RT-PCR and the products were resolved by electrophoresis in agarose gels.

M - 100bp DNA ladder,
Lane 1 - Wild type brine shrimp larvae,
Lane 2 - Brine shrimp larvae injected with dsRNA *gfp*
Lane 3 - Brine shrimp larvae injected with dsRNA *hsp70*
Lane 4 - Wild type brine shrimp larvae pretreated with phloroglucinol
Lane 5 - Brine shrimp larvae injected with dsRNA *gfp* and pretreated with phloroglucinol,
Lane 6 - Brine shrimp larvae injected with dsRNA *hsp70* and pretreated with phloroglucinol

Kumar et al. 2018. Frontiers in Immunology. 9:1091

Mode of Action of Phloroglucinol



Phloroglucinol

+

Damage Control System (Hsp70)

-

Reduce virulence factor production in *Vibrio*

Kumar et al. 2018. Frontiers in Immunology. 9:1091

Anti-*Vibrio* Effect of Phloroglucinol – Validation Study Ongoing

The Health Beneficial Activities of Tex-OE®

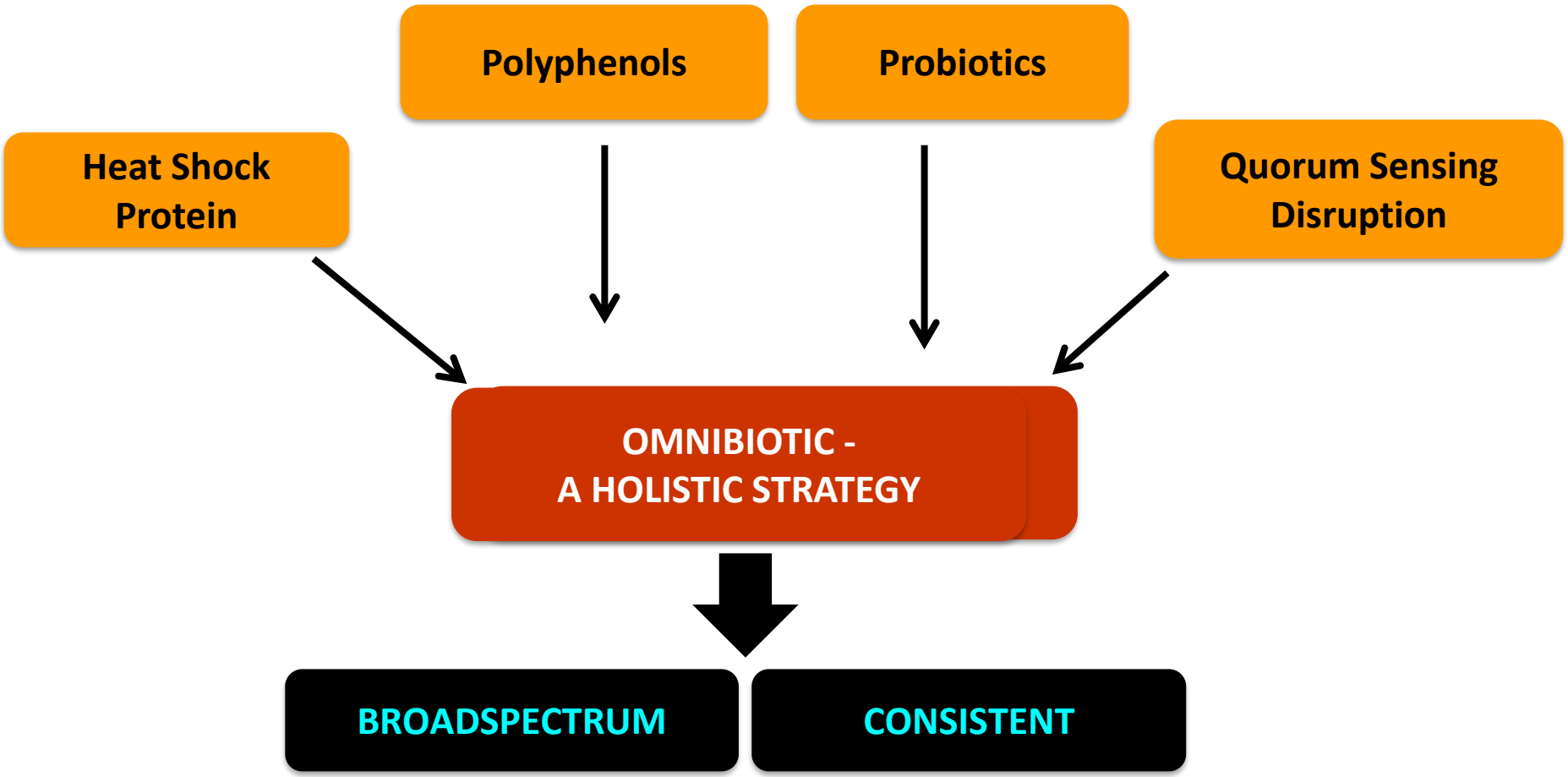
Findings Snapshot

- Temperature stress
- Osmotic shock
- *V. campbellii*
- *V. harveyi*

Baruah et al. (2012) Aquaculture 334-337:152-158

Baruah et al. (2014) Dev & Comp Immunol 46:470-9

FUTURE PERSPECTIVES



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