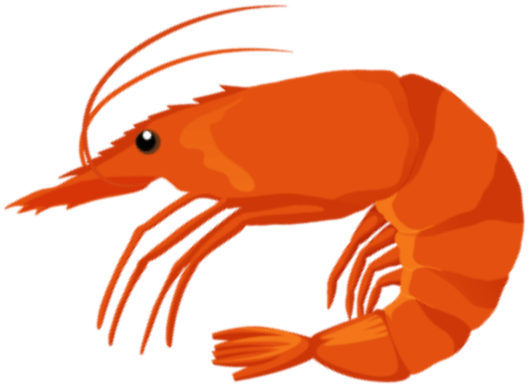
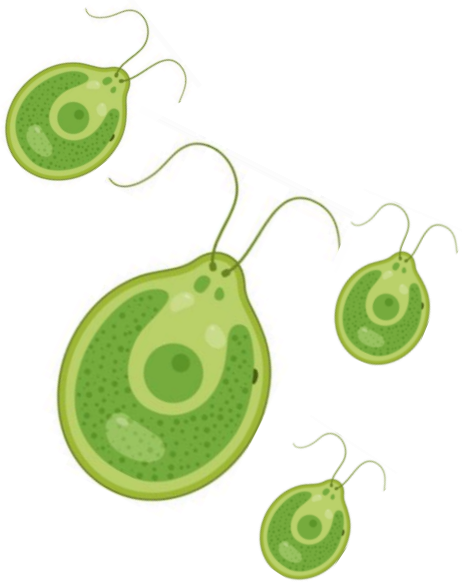


# Shrimp antiviral vaccine

## By generation of microalga chloroplast expressing dsRNA

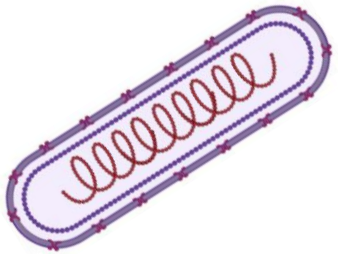



Presented By:

**Afnan Khaled**


Research Topics and Seminar (510)

23rd Nov, 2021

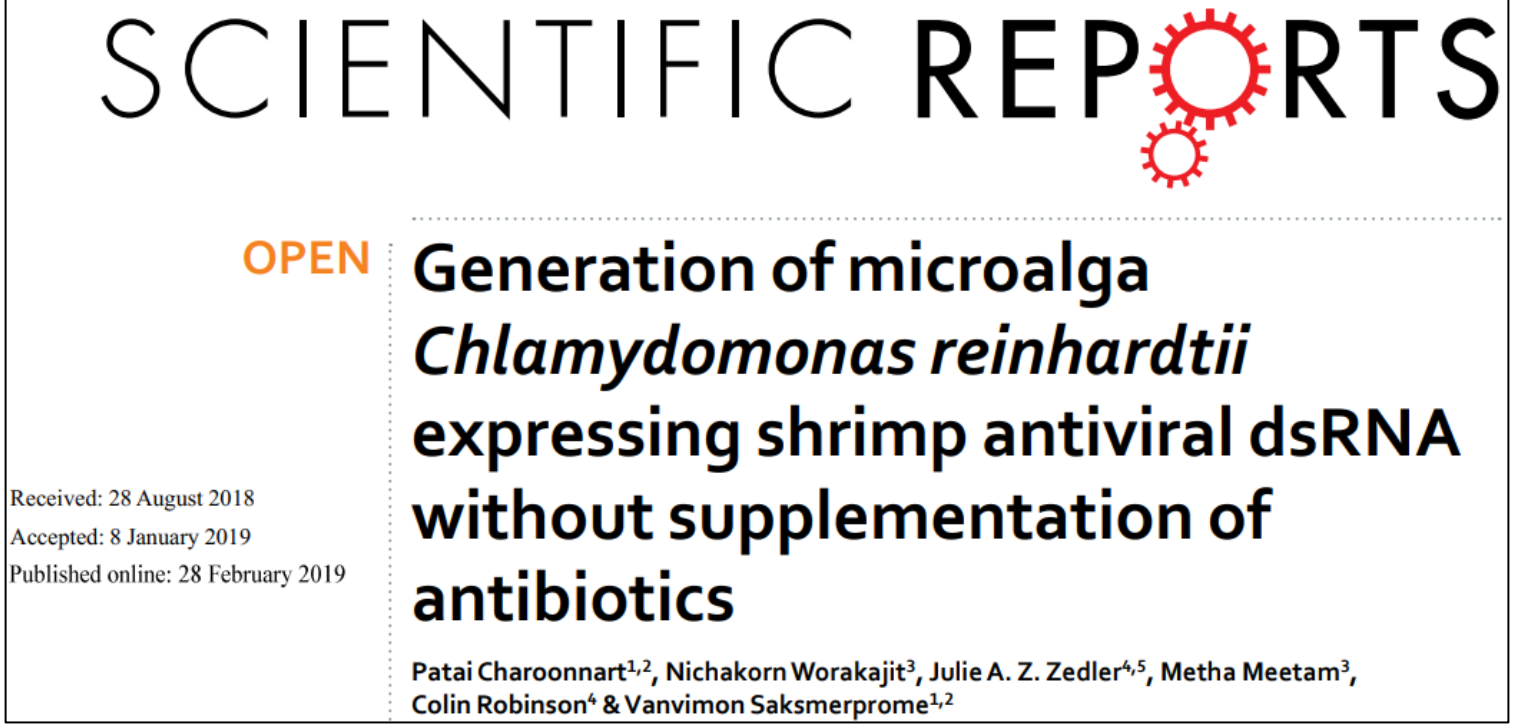


# Outline

- The selected paper
- Why this paper? A brief background
- RNAi and previous applications in shrimps
- Paper objectives and abstract
- Methodology and Results
- Conclusion
- Possible applications in Middle EAST

# The selected paper

www.nature.com/scientificreports




# What are the organisms?

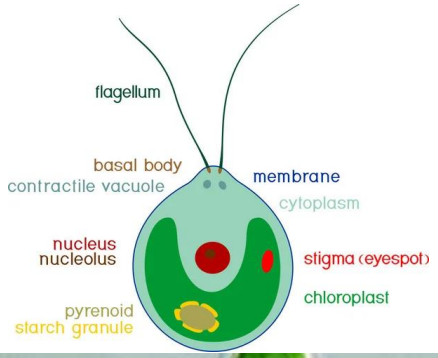
## White leg shrimp

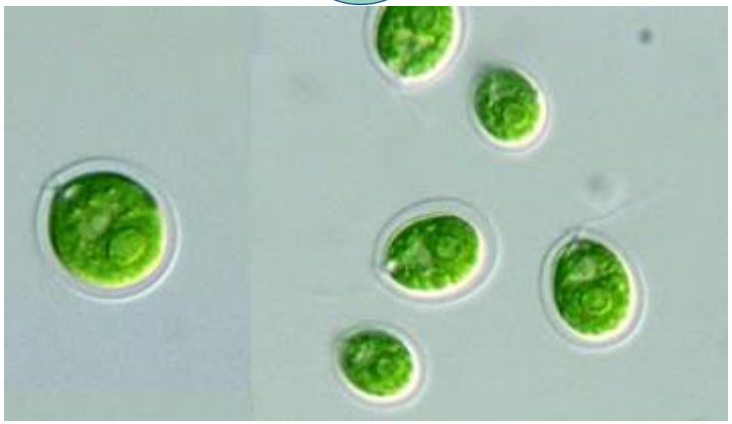
*Penaeus vannamei*

Can feed on

## Green Microalga

*Chlamydomonas reinhardtii*

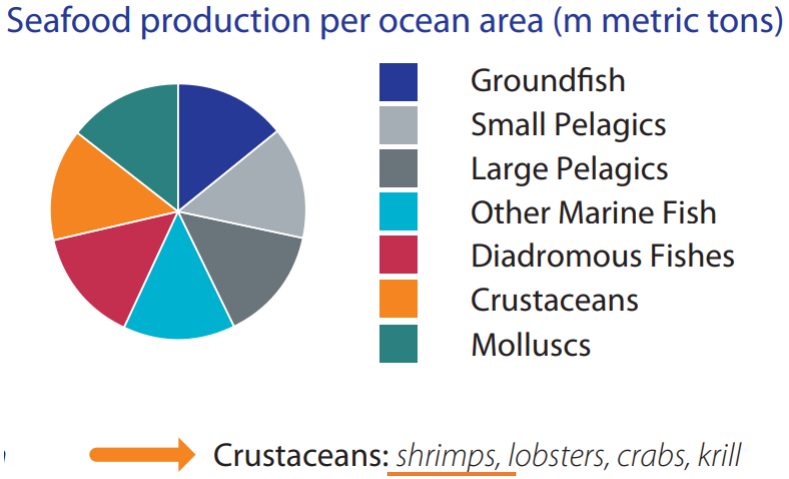


# Why this paper?

- Shrimp covers 15% of total seafood products.

- Shrimp farming exceeds 55% of global shrimp production, Mainly from China and Thailand.




World seafood Map 2017, Rabobank

Shrimp farming (Aquaculture)

# Why this paper?

- Several outbreaks reported from **Viral infections**

  Most threat to shrimp farming - Spread fast - High mortality rate

**White spot prawn disease now endemic to wild populations in Queensland's Moreton Bay region**

ABC Radio Brisbane / By Josh Edwards and Antonia O'Flaherty

Posted Fri 26 Feb 2021 at 12:56pm

NIKKEI Asia

News by Location | Business | Markets | Tech | Politics | Economy | Features | Opinion | Life & Arts

TURBULENT THAILAND

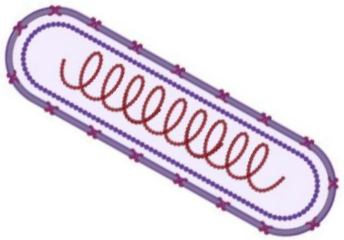
Thai protest demands help for shrimp sellers after virus outbreak

Anti government protesters sell shrimps in front of government house as people now fear to eat shrimps due to the coronavirus disease (COVID-19) outbreak in Bangkok on Dec. 26. © Reuters

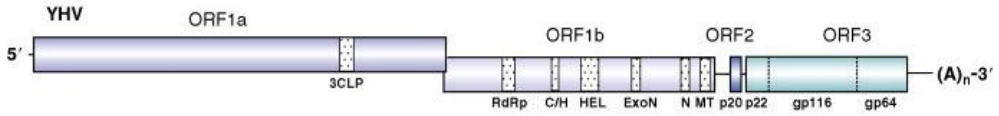
December 26, 2020 19:44 JST

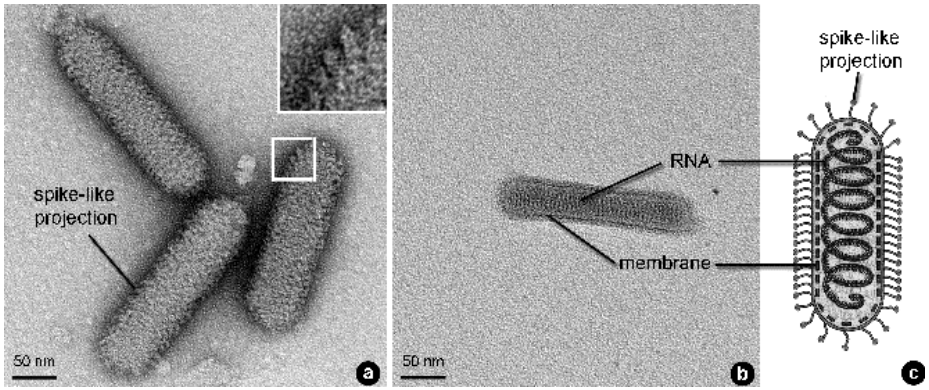
| Disease | Causative agent | Geographical distribution |
|---|---|---|
| White spot disease | White spot syndrome virus, a dsDNA virus | Asia, America, Middle East, Europe, Africa |
| Yellow head disease | Yellow head virus and Gill associated virus (GAV), ssRNA virus (Yellow head virus complex has six genotypes and GAV belongs to genotype 2) | Asia, Pacific, and Australia |
| Infectious hypodermal and hematopoietic necrosis (IHHN) | IHHN virus, a ssDNA virus | Worldwide |
| Taura syndrome | Taura syndrome virus, a ssRNA virus | Americas, parts of Asia |
| Infectious myonecrosis | Infectious myonecrosis virus (IMNV), a dsRNA virus | Americas, parts of Asia |

# Why this paper?



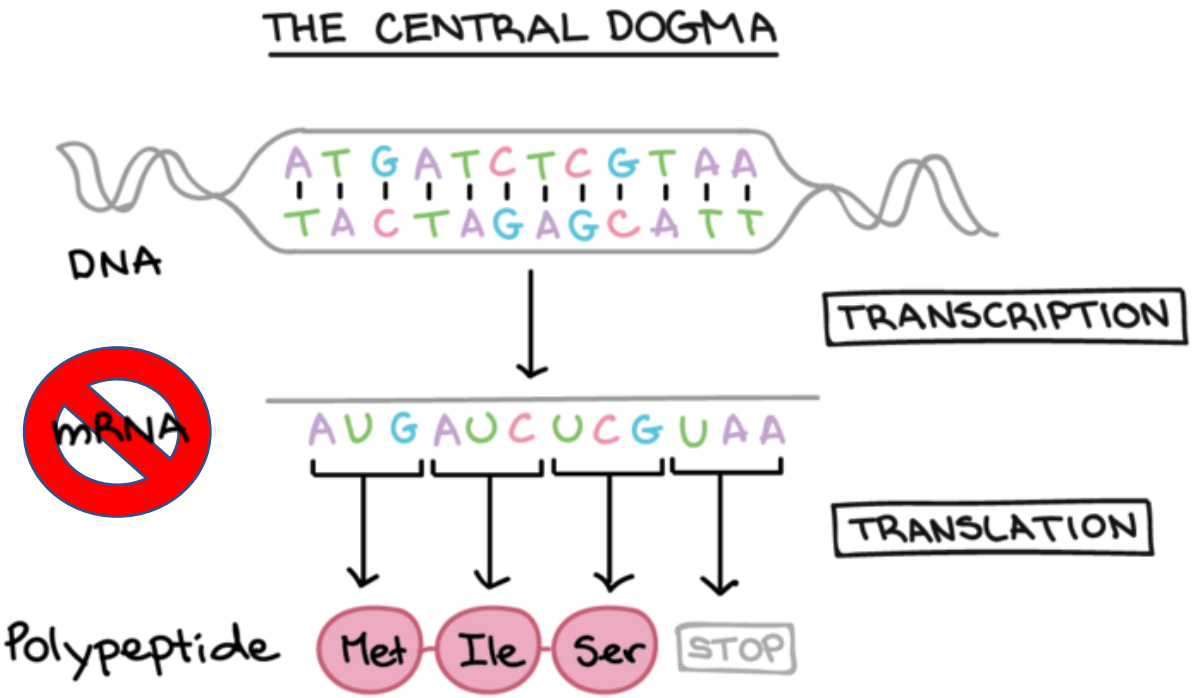
- **Yellow Head Virus** (YHV)
  - ssRNA
  - highly virulent pathogen that can cause 100% mortality within a few days of the first signs of disease in a pond.



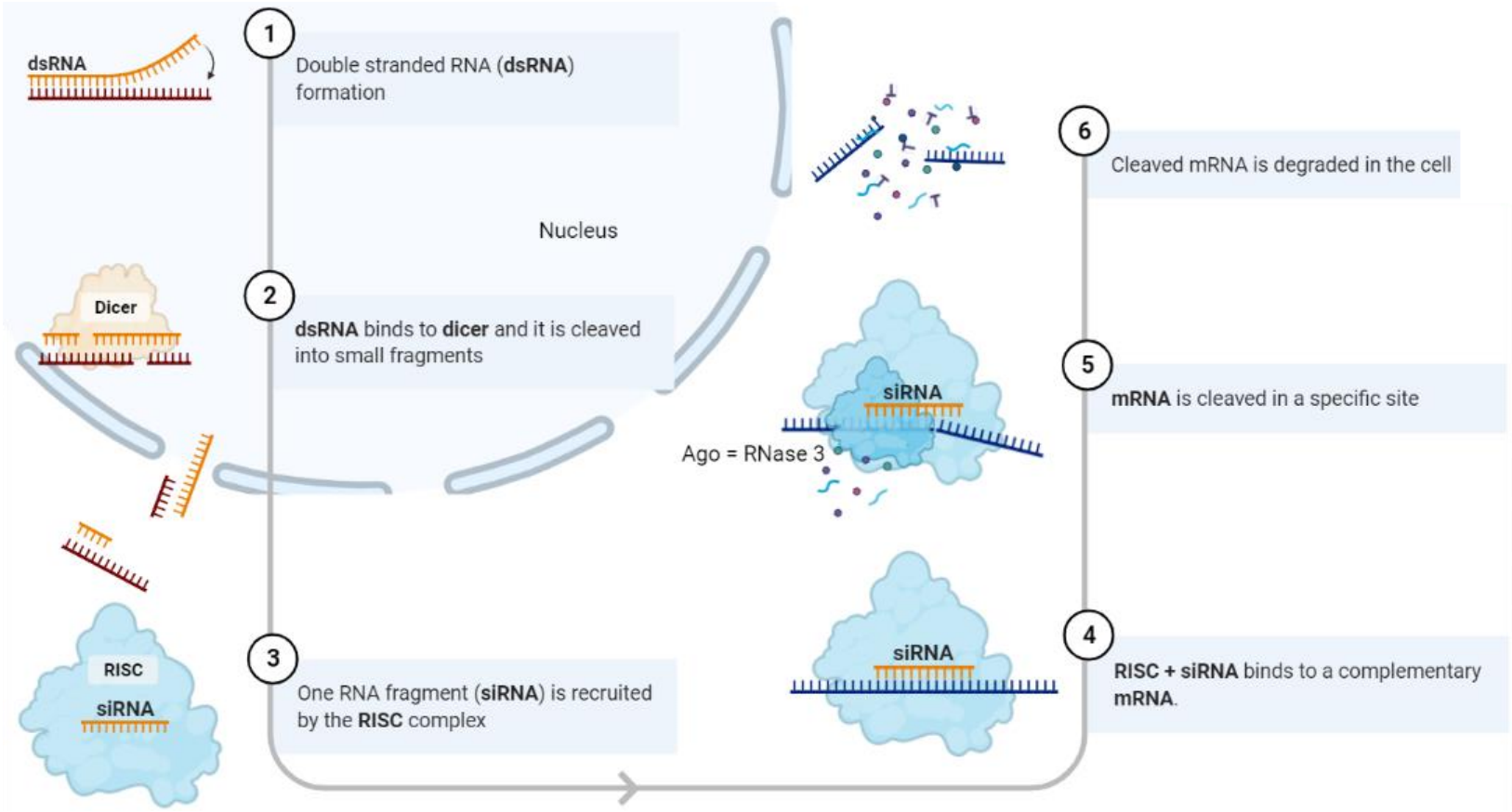

# RNAi mechanism

- **Interfering RNAs**, small dsRNA that direct sequence specific mRNA degradation.



# RNAi mechanism

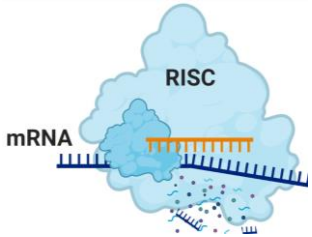
- **Interfering RNAs**, small dsRNA that direct sequence specific mRNA degradation.



# Paper Objective and Abstract

RNA interference (RNAi) is an effective way of combating shrimp viruses by using sequence-specific double-stranded (dsRNA) designed to knock down key viral genes. The aim of this study was to use microalgae expressing antiviral dsRNA as a sustainable feed supplement for shrimp offering viral protection. In this proof of concept, we engineered the chloroplast genome of the green microalga *Chlamydomonas reinhardtii* for the expression of a dsRNA cassette targeting a shrimp yellow head viral gene. We used a previously described chloroplast transformation approach that allows for the generation of stable, marker-free *C. reinhardtii* transformants without the supplementation of antibiotics. The generated dsRNA-expressing microalgal strain was then used in a shrimp feeding trial to evaluate the efficiency of the algal RNAi-based vaccine against the virus. Shrimps treated with dsRNA-expressed algal cells prior to YHV infection had 50% survival at 8 day-post infection (dpi), whereas 84.1% mortality was observed in control groups exposed to the YHV virus. RT-PCR using viral specific primers revealed a lower infection rate in dsRNA-expressing algae treated shrimp (55.6 ± 11.1%) compared to control groups (88.9 ± 11.1% and 100.0 ± 0.0%, respectively). Our results are promising for using microalgae as a novel, sustainable alternative as a nutritious, anti-viral protective feedstock in shrimp aquaculture.

# RNAi and previous applications in shrimps

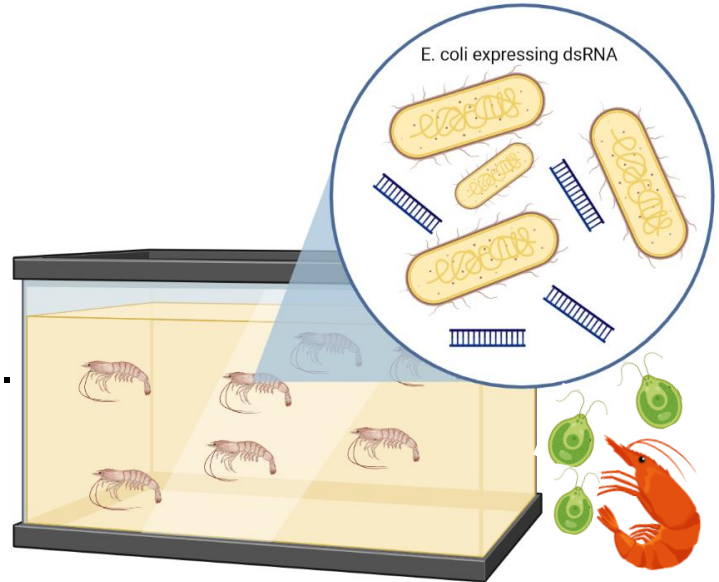



- Is the tool new?

  No!

The shrimp aquaculture industry remains threatened by significant losses due to several viral pathogens such as the yellow head virus (YHV) and the white spot syndrome virus (WSSV). RNA interference (RNAi) technology is a novel, highly effective technology for combating viral pathogens by using sequence-specific double-stranded (dsRNA) designed to knockdown the key viral genes. This method aims to harness the natural abilities of animals to combat pathogen infections. The application of this technology in small-scale tests suggests high efficiency of RNAi in controlling shrimp viruses that cause high mortality rates and slow growth syndrome[1,2]. For large-scale production, **the utilization of a RNase III-deficient *Escherichia coli* strain expressing gene-specific dsRNA is efficient and inexpensive**[1]. However, regular use of *E. coli* cells in shrimp feed has yet to be investigated for its **long-term effect on the animals and the environment.** Thus, finding alternative dsRNA production and delivery systems that can be safely used in shrimp farms is a key challenge for future application of this technology.

- **Microalga** is more advantages than E.*coli*:
  1. used before as cell factory.
  2. generally regarded as Safe.
  3. well studied nuclear & chloroplast genome.
  4. Easy delivery.



# RNAi and previous applications in shrimps

- Is the organism new?

  No!

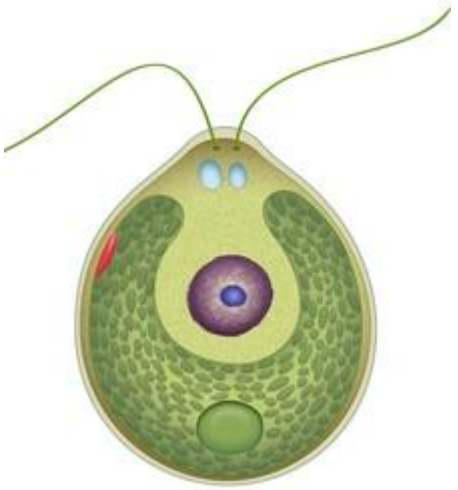
The potential of dsRNA produced from the nuclear genome of *C. reinhardtii* to protect shrimps from YHV infection was previously shown[7]. Meanwhile, the chloroplast has become an attractive target for dsRNA production since it lacks any RNAi machinery for dsRNA processing allowing high-level accumulation of dsRNA within the organelle[8]. In addition, chloroplast transformation in *C. reinhardtii* occurrs via homologous recombination leading to integration of the gene of interest in a specific site whereas integration into the nuclear genome occurs randomly causing instability and issues with gene silencing[9]. More importantly, chloroplast transformation can be achieved by utilizing non-photosynthetic mutant strains, allowing for the generation of marker-free transformants that do not require antibiotics for selection[10].

- **Chloroplast genome** is more advantageous than nuclear genome:

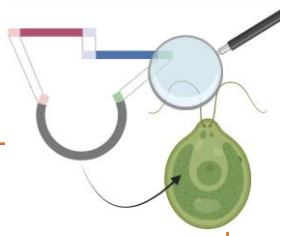
  **1**. lacks RNAi machinery.

  **2**. Insertion of target at specific site by homologous recombination.

  **3**. marker free selection.

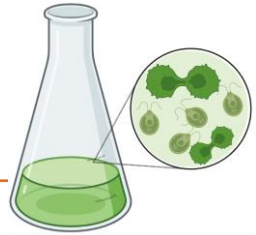


# Methodology Outline



**1**

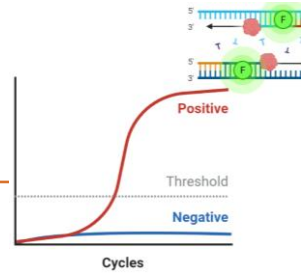
Cloning of dsRNA cassette in chloroplast, transformation and PCR analysis



**2**

Growth Analysis of transformed microalgae

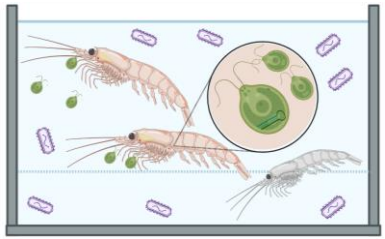
(recombinant vs. non recombinant)




**3**

Check dsRNA expression & Quantification

(RT-PCR, RT-qPCR)



**4**

Shrimp feeding and Survival test

# Method and Results (1)

1

Cloning of dsRNA cassette in chloroplast, transformation and PCR analysis

- Culturing of non-photosynthetic Microalga strain “TN72”
  - ➢ Mutant -lacks functional psbH gene


Order plasmid

Another plasmid suitable for TN72

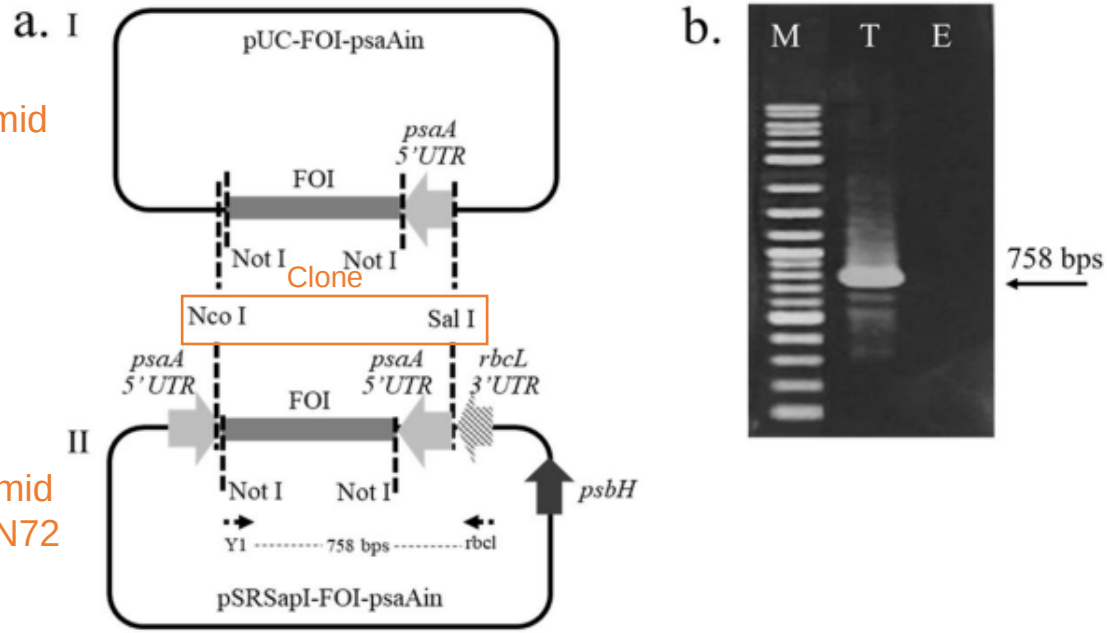


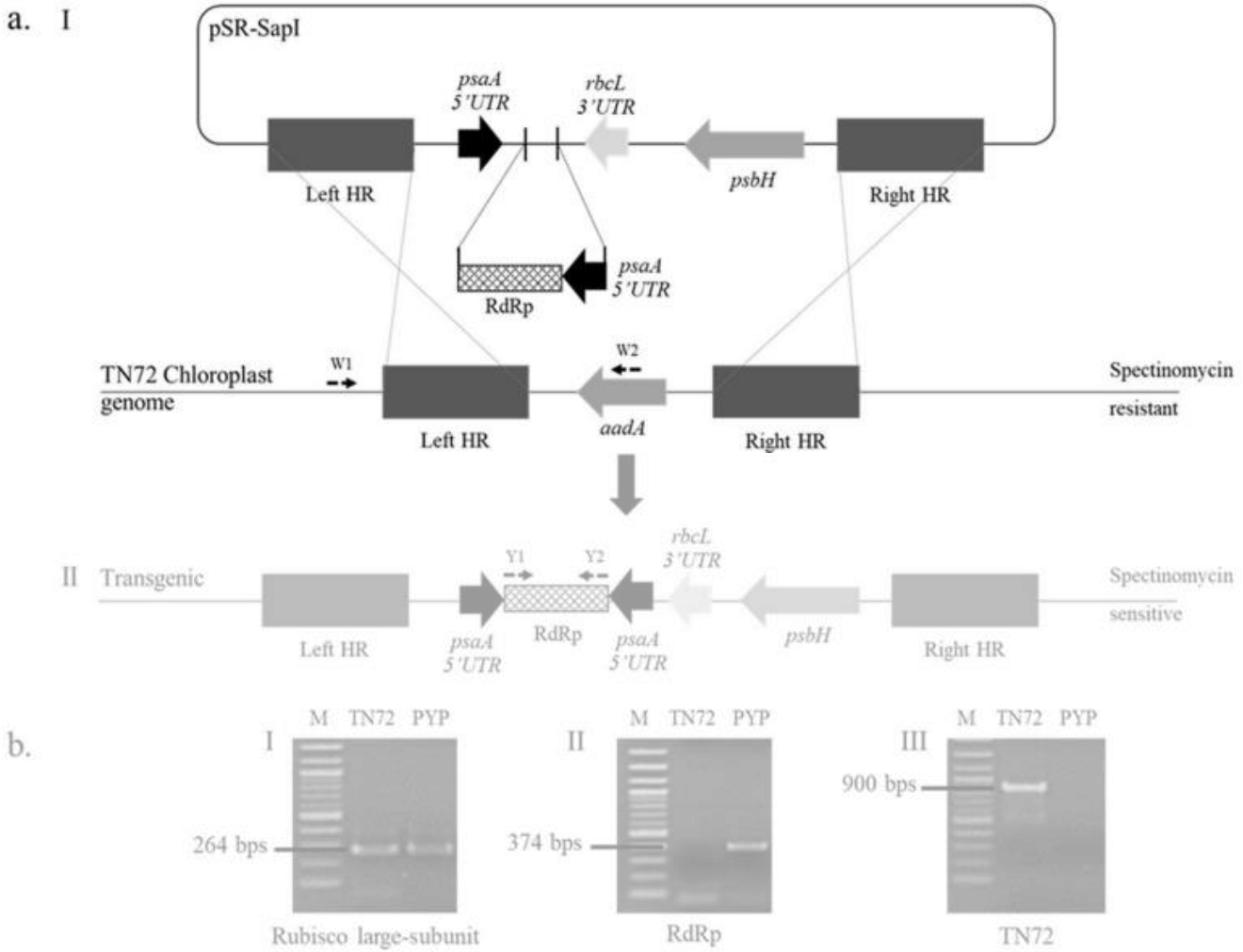

**Figure 1.** (**a**) Construction procedure of convergent promoter cassette expressing dsRNA. The cloning of fragment-of-interest (FOI) linked with an inverted *psaA* promoter (pUC-YHV_RdRp-psaAin) (I) into pSRSap I generating recombinant plasmid for chloroplast transformation (pSRSapI-FOI-psaAin) (II). (**b**) PCR analysis confirming insertion and direction of FOI-psaA in pSRSap I using primers Y1 and rbcl illustrated on the left whereas lane M is 2-log DNA marker, lane T represents pSRSapI-FOI-psaAin (pSR-PYP), and lane E represents pSRSapI.

# Method and Results (1)

1

Cloning of dsRNA cassette in chloroplast, transformation and PCR analysis

- Transformation inside chloroplast

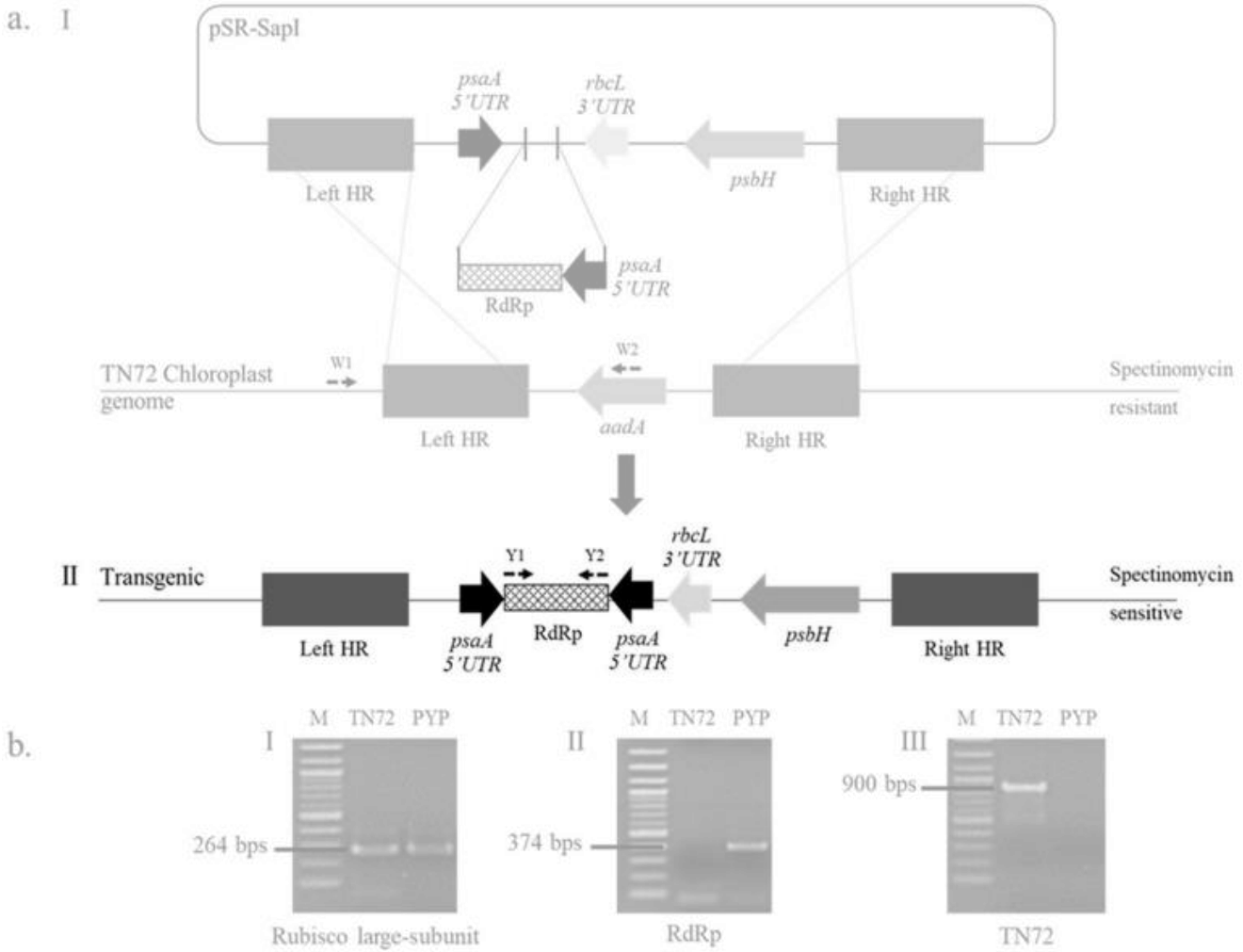


# Method and Results (1)

1

Cloning of dsRNA cassette in chloroplast, transformation and PCR analysis

- Transformation inside chloroplast

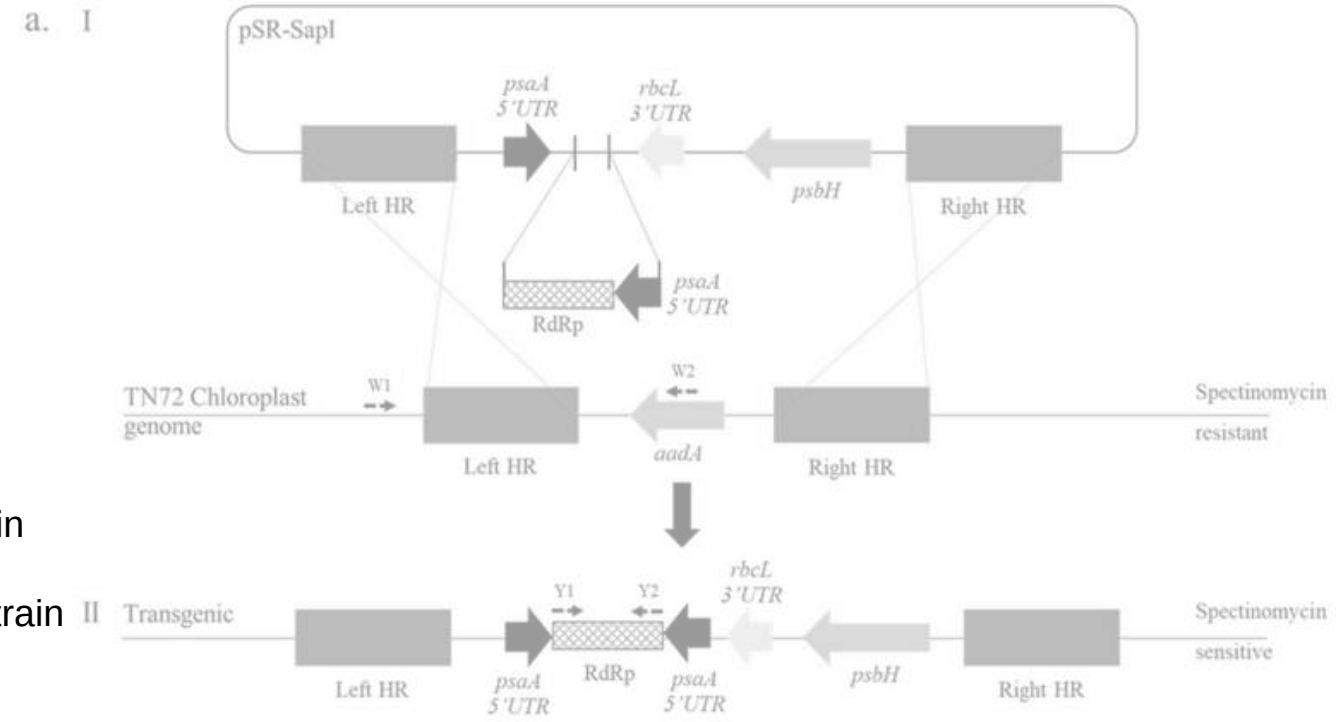


# Method and Results (1)

1

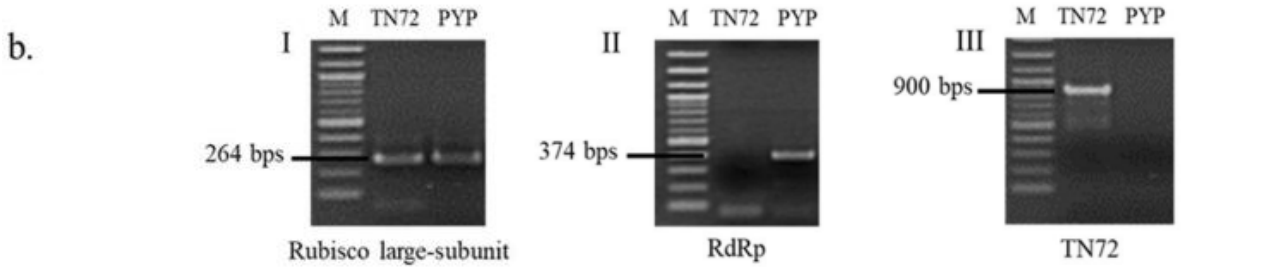
Cloning of dsRNA cassette in chloroplast, transformation and PCR analysis

- Transformation inside chloroplast




**TN72**: control strain

**PYP**: generated strain




**Control**
Housekeeping gene

**Successful Integration**
Inserted target gene

**Confirm Homoplasmy**
By W1 and W2 primers

# Method and Results (2)

2
Growth Analysis of transformed microalgae
(recombinant vs. non recombinant)

- Growth analysis of transformed chloroplast (SR vs. PYP)

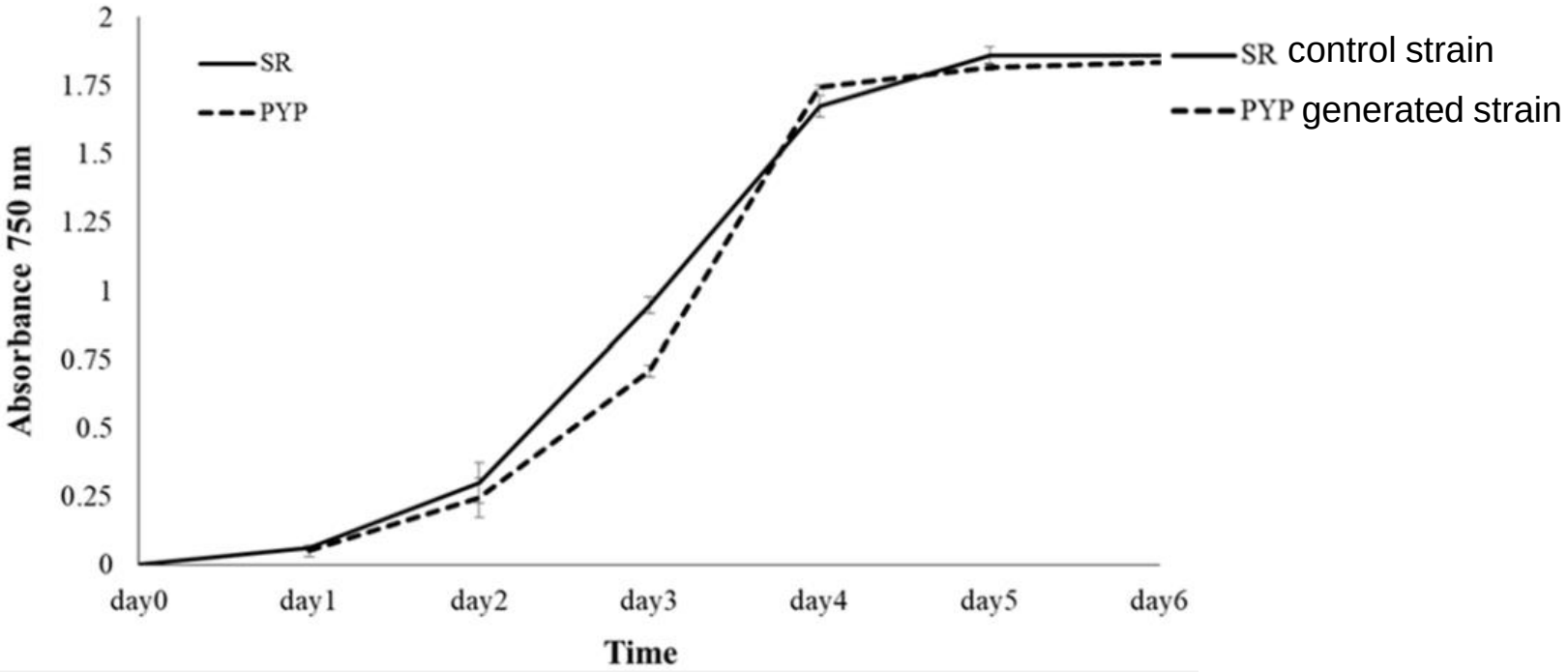
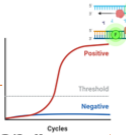



**Figure 3.** Growth rate according to absorbance at wavelength 750 nm of dsRNA-expressing *C. reinhardtii* strain (PYP,--) comparing to the control strain (SR, -). Bar represents standard deviation.

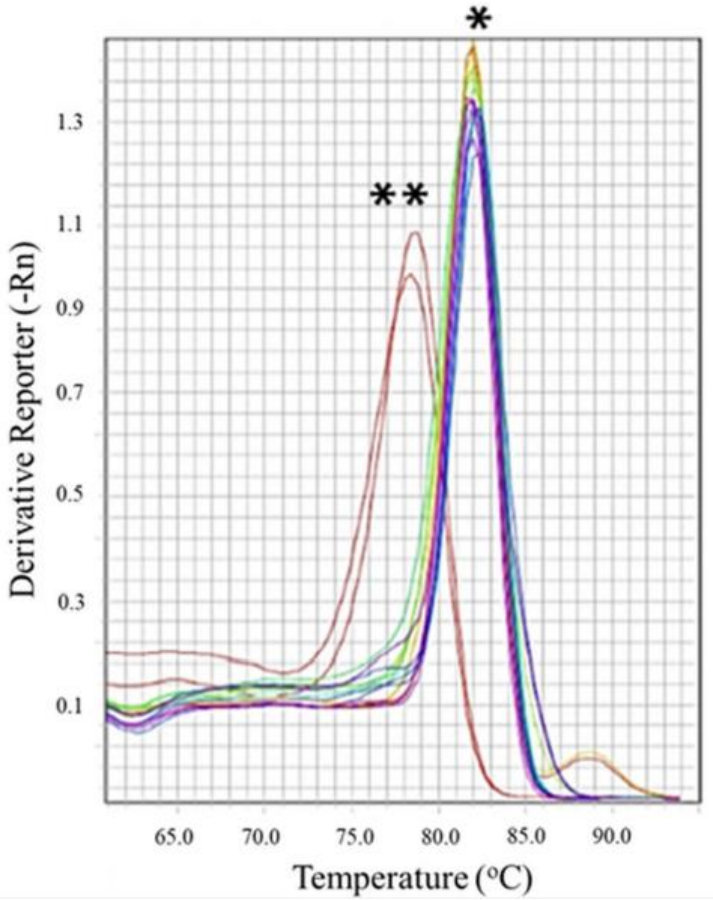
Expression of introduced cassette does not affect microalga growth rate

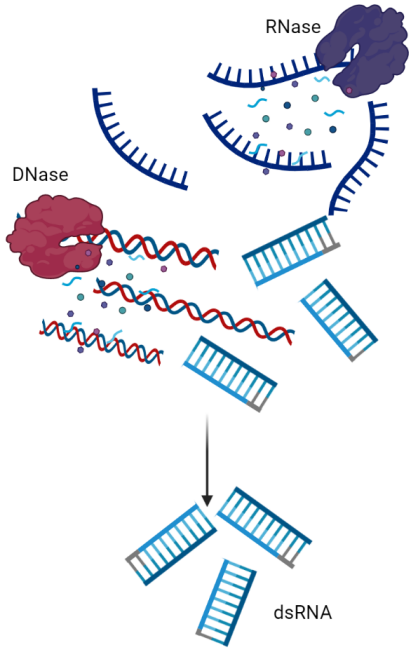
# Method and Results (3)



3
Check dsRNA expression & Quantification
(RT-PCR, RT-qPCR)

- Test dsRNA expression from PYP (generated strain) by RT-qPCR

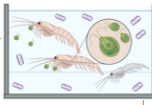



Yield of dsRNA in PYP is very near to standard E.coli, but relatively lower

**Figure 4.** Confirmation of dsRNA by RT-qPCR by *represents melting temperature ($T_m$) of dsRNA-YHV expressed from *E. coli* HT115 and dsRNA-YHV extracted from PYP strain while **represents negative reaction using $ddH_2O$ as a template.

# Method and Results (4)



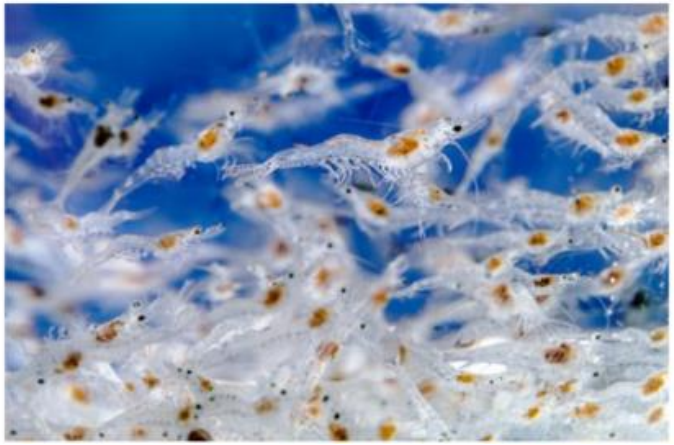
4

Shrimp feeding and Survival test

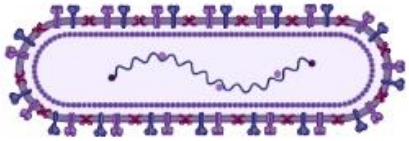
- Shrimp Feeding and viral challenge

Feeding

post larve stage



Challenge



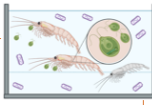
Observation for 8 days

% of survival

Semiquantitative RT-PCR after 8 days

Gel electrophoresis (different viral gene vs. Actin gene)

# Method and Results (4)



4

Shrimp feeding and Survival test

- Observation for 8 days

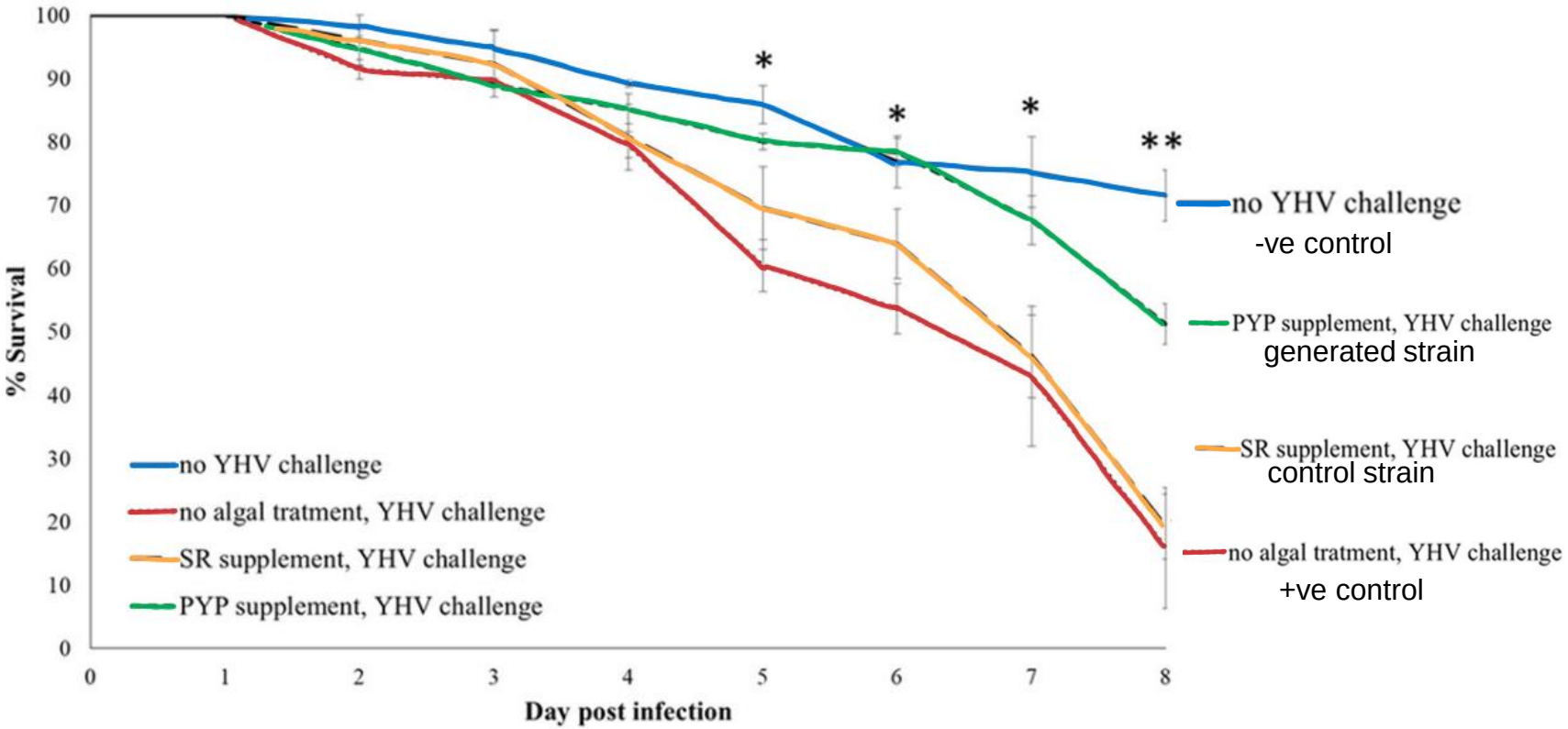
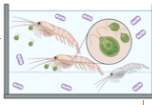



**Figure 5.** Post-larval shrimp survival percentage from feeding trial comparing between observed group (no YHV challenge) and normal feed (YHV challenge), SR supplement prior YHV challenge, and PYP supplement prior YHV oral challenge. Bar represents standard error. *and **Represent significant difference between experiment groups within observation day at confidential level $p < 0.05$ and $p < 0.01$, respectively.

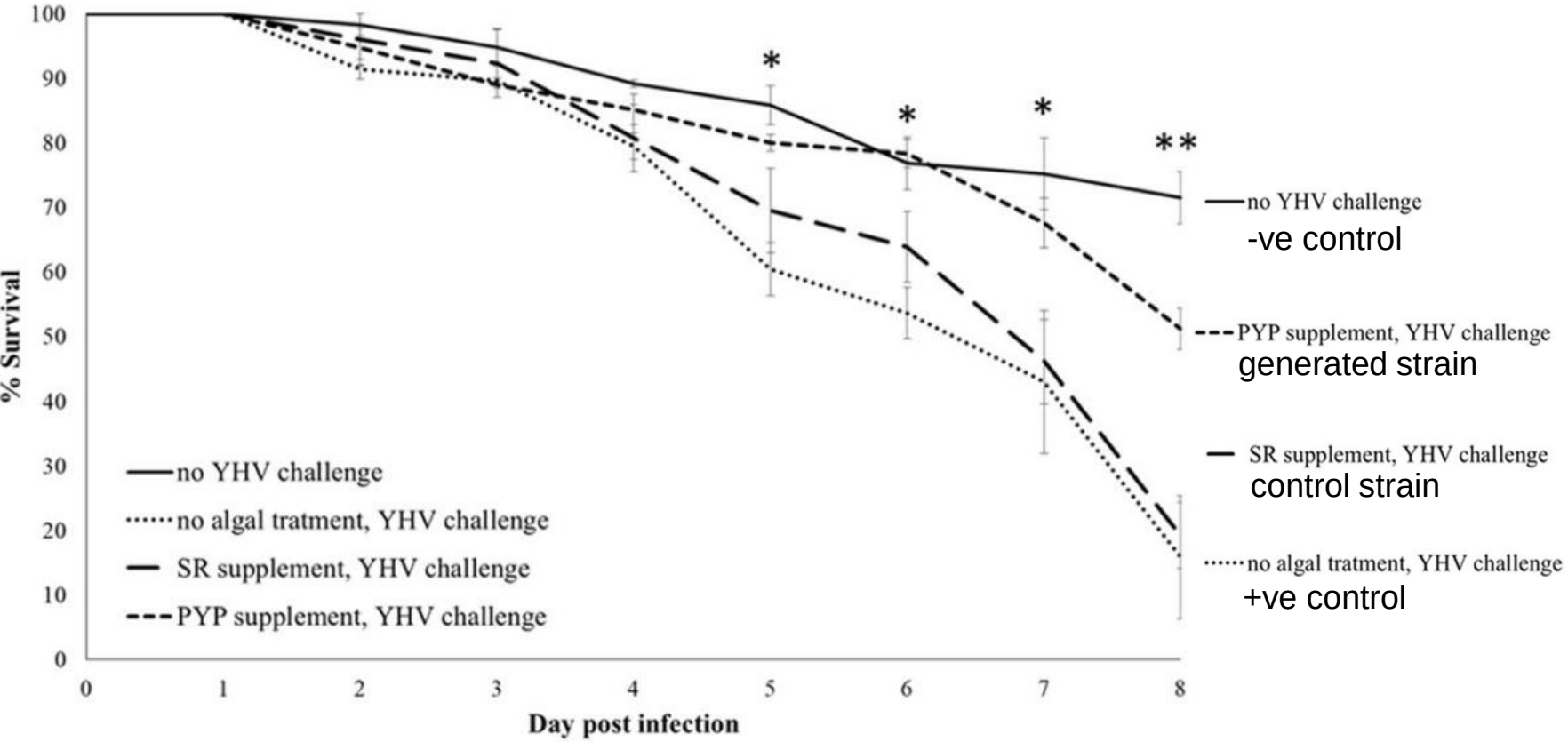
# Method and Results (4)



4

Shrimp feeding and Survival test

- Observation for 8 days

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# Method and Results (4)



- Semiquantitative RT-PCR after 8 days

No YHV challenge

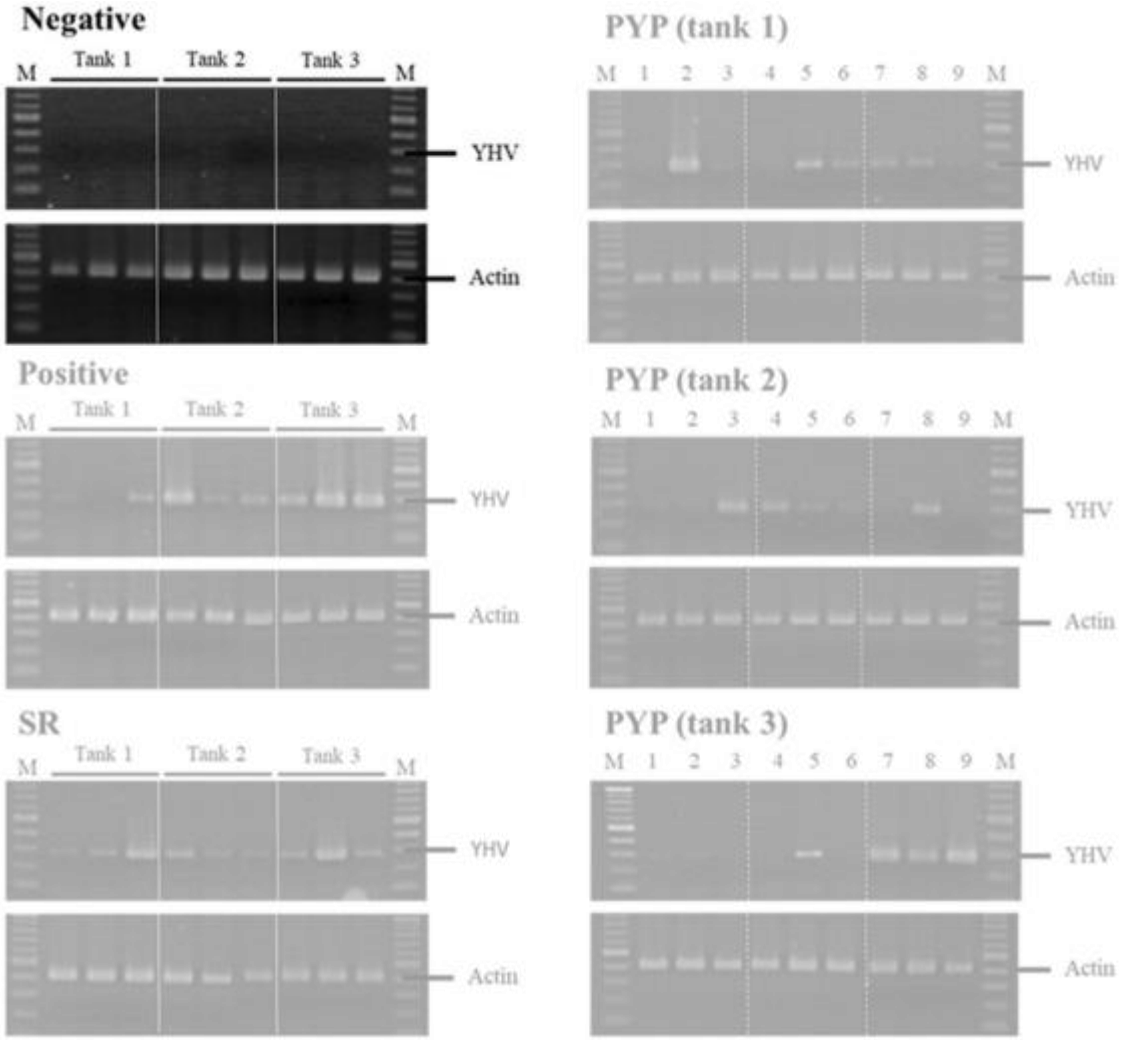



**Figure 6.** RT-PCR analysis detecting YHV infection of survival shrimps at 8 d.p.i. Actin specific primers were use as internal control. Lane M is 2-log DNA marker. Each lane represents infection level of individual PL shrimp. Solid lines separate 3 individual shrimps in each tank. Dash lines separate 3 individual shrimp in the same tank. Noted that there were more shrimps remaining in negative group while all remaining shrimp in the other groups was analyzed and presented in this figure.

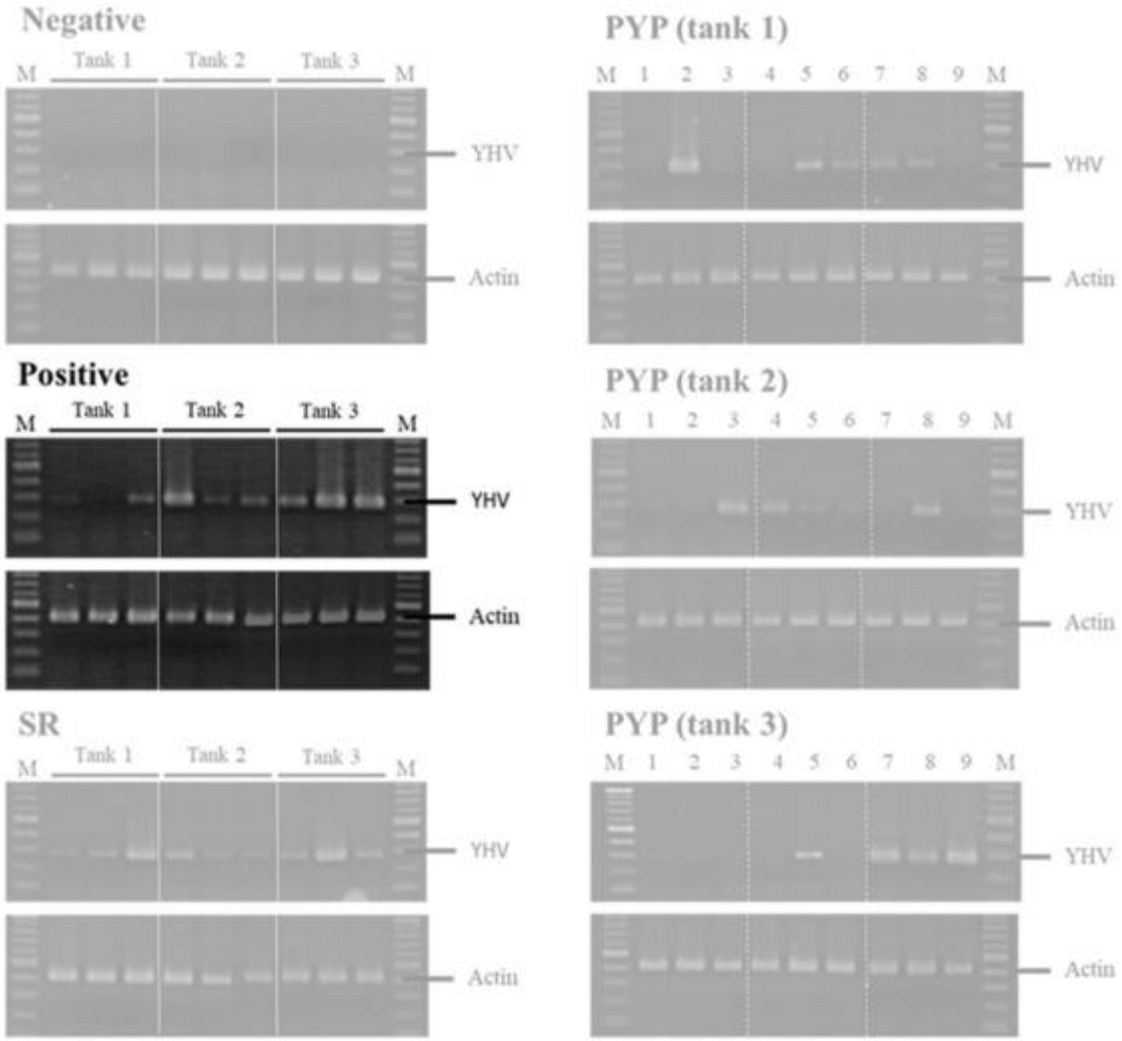
# Method and Results (4)



- Semiquantitative RT-PCR after 8 days

No YHV challenge

No algal treatment, YHV challenge

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# Method and Results (4)

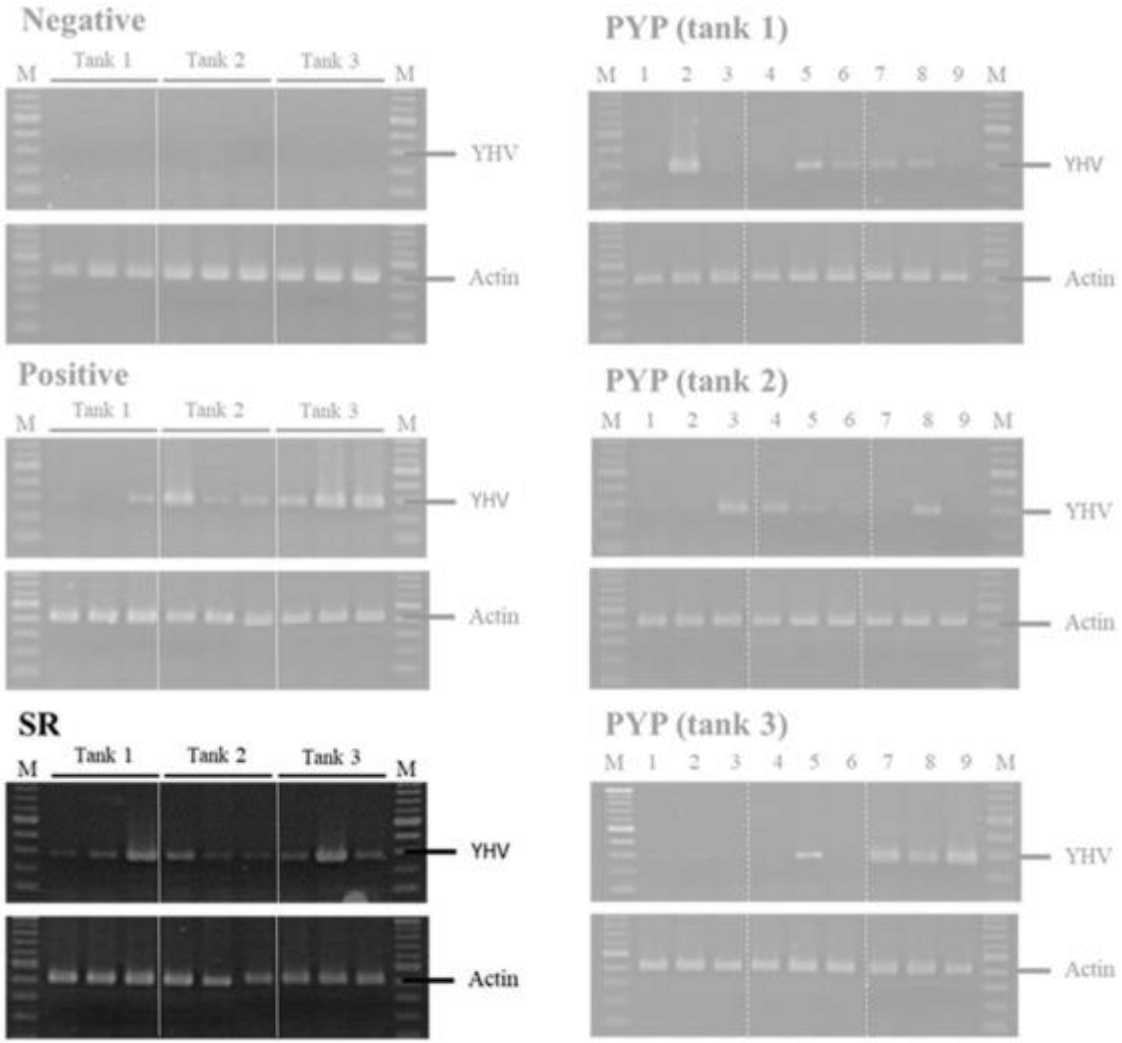


- Semiquantitative RT-PCR after 8 days

No YHV challenge

No algal treatment, YHV challenge

Control strain, YHV challenge

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# Method and Results (4)



- Semiquantitative RT-PCR after 8 days

No YHV challenge

No algal treatment, YHV challenge

Control strain, YHV challenge

PYP Generated Strain

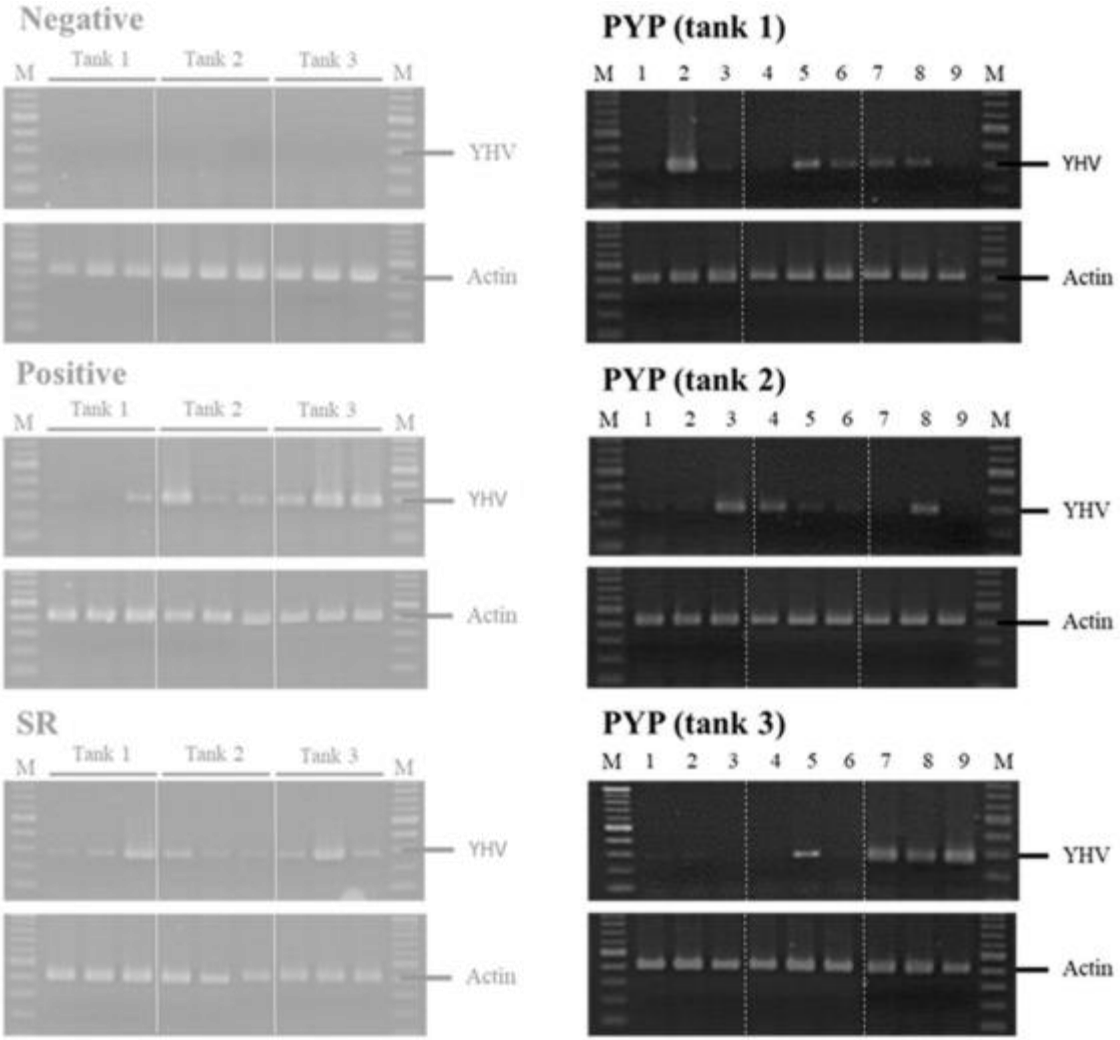



**Figure 6.** RT-PCR analysis detecting YHV infection of survival shrimps at 8 d.p.i. Actin specific primers were use as internal control. Lane M is 2-log DNA marker. Each lane represents infection level of individual PL shrimp. Solid lines separate 3 individual shrimps in each tank. Dash lines separate 3 individual shrimp in the same tank. Noted that there were more shrimps remaining in negative group while all remaining shrimp in the other groups was analyzed and presented in this figure.

# Method and Results (4)

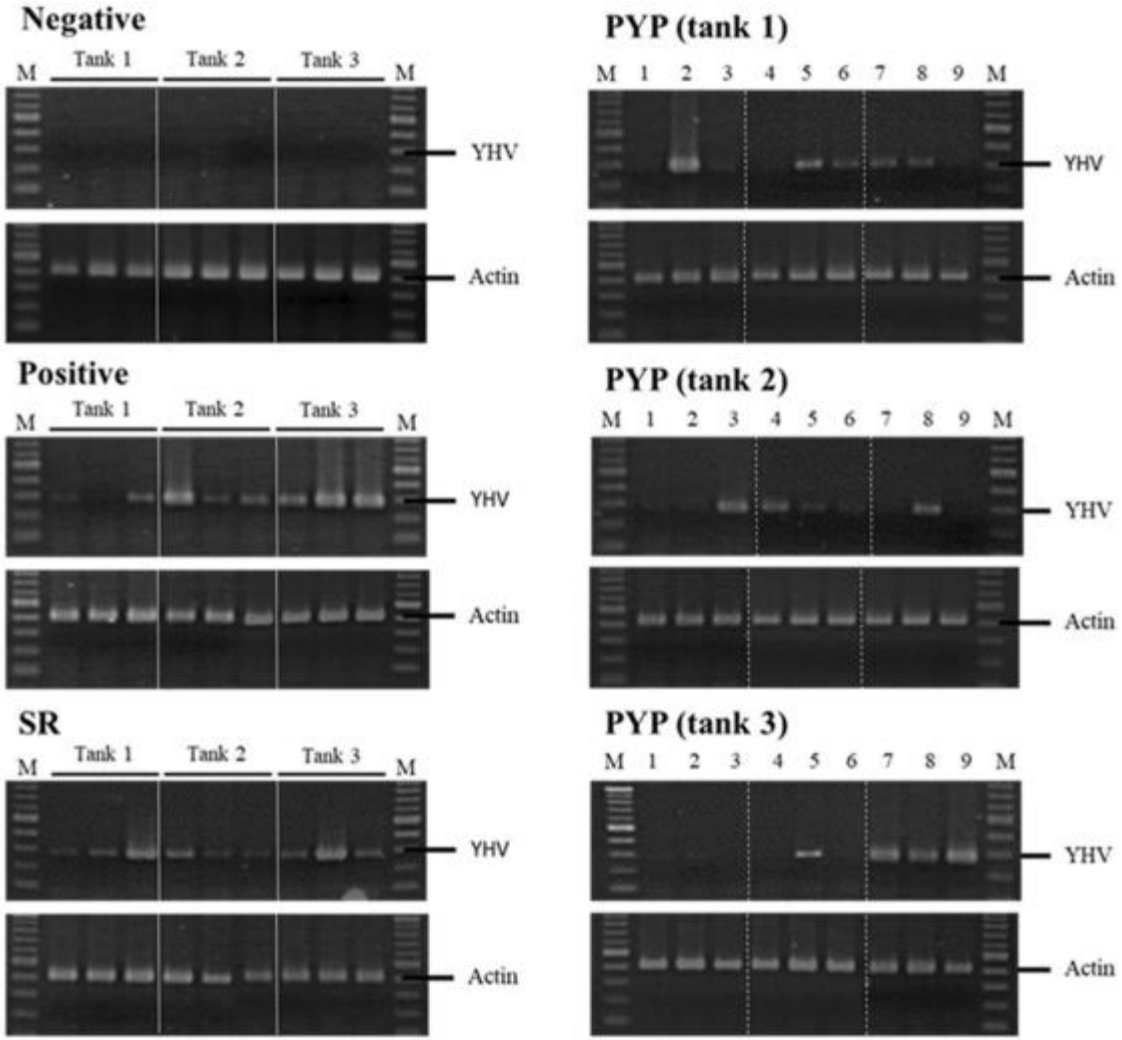


- Semiquantitative RT-PCR after 8 days

No YHV challenge

No algal treatment, YHV challenge

Control strain, YHV challenge




PYP Generated Strain

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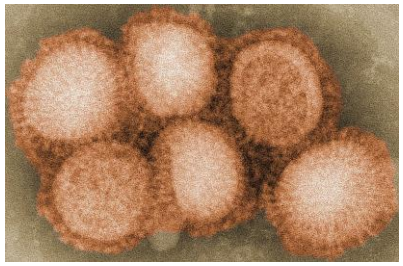
# Conclusion

- Shrimps showed significant resistance against viral infection after processing dsRNA that expressed and delivered orally through microalga’s chloroplast.
- Future studies needed to address public concerns on using living genetically modified organisms in aquaculture feed, hence algal cell should be inactivated before supplementation until approval.

# Possible applications in Middle East

- Introducing RNAi system in bird's food (grass or worms) to vaccinate them against viral infections.
  - Such as: **Avian influenza!**



# Thanks for Listening!

Another personal aim of the study 😋