

# DEVELOPMENT OF NEW AQUACULTURE AND SEED PRODUCTION TECHNOLOGY FOR COMMERCIALY-IMPORTANT SHRIMP SPECIES: RESEARCH AT JIRCAS

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**Bong Jung Kang** serves as Research Scientist at the Japan International Research Center for Agricultural Sciences (JIRCAS). She received an undergraduate degree in Biology from Dong-Eui University (South Korea), and a Ph.D. in Biomolecular Sciences from Okayama University, and after which she conducted postdoctoral study at Okayama University and JIRCAS prior to her current position. Dr. Kang previously conducted research on the elucidation of vitellogenin structure pertaining to reproductive mechanisms in crustaceans and fish. Currently, her research focuses on the development of new technology for the promotion of shrimp maturation in captivity using economically-important prawn/shrimp species. She has authored numerous research papers in her field, and has recently provided an entry for the authoritative Encyclopedia of Reproduction (2nd Edition), which is published only once every twenty years.



# ABSTRACT

Shrimp culture is a significant component of the world's fisheries industry, and is conducted widely in South/Southeast Asian countries. Penaeid shrimp species are mainly targeted in such endeavors, and among these, the whiteleg shrimp, *Litopenaeus vannamei*, constitutes more than 80% of the world's total production. In order to support this very large shrimp farming industry, it is essential to produce sufficient quantities of juvenile shrimp. To this end, eyestalk ablation is frequently used in commercial hatcheries in order to induce ovarian maturation and spawning. However, eyestalk ablation exhibits adverse effects, such as increased mortality rates and deterioration of egg quality; moreover, it has received a great deal of criticism from the viewpoint of animal welfare by the general public. Therefore, it is urgent to develop a means of promoting maturation based on the understanding of the physiological functioning of the animal, in order to establish a more effective means of seed production for species such as *L. vannamei*.

With the goal of contributing to the promotion of a more sustainable shrimp farming industry, the Japan International Research Center for Agricultural Sciences (JIRCAS) is implementing a research project entitled "Development of advanced seed production and culture technology for economically-important shrimp species based on elucidation of reproductive mechanisms". This project is being carried out with the aim of elucidating mechanisms of maturation, with the ultimate goal of developing reproductive-stimulating technology that could potentially replace eyestalk ablation.

Many aspects of crustacean reproduction and the associated mechanisms in shrimp remain unclear; however, it is well-known that ovarian maturation is regulated negatively by neurohormones such as vitellogenesis-inhibiting hormone (VIH). VIH is synthesized at the X-organ/sinus gland complex, a neurosecretory organ located in the eyestalks. It is then secreted into the hemolymph, after which it acts negatively on reproduction by inhibiting vitellogenin (Vg) synthesis. Vg, which is the precursor of the major egg yolk protein in oviparous animals, is synthesized at the hepatopancreas and ovary, secreted into the hemolymph, and then accumulated in developing oocytes.

Studies at JIRCAS have utilized *L. vannamei* as a model animal; the principle aim of research up to now has been focused on acquiring basic information necessary for understanding reproductive mechanisms in further detail. For example, we have developed measurement systems for VIH and Vg, and have examined their relative dynamics in context of molting and eyestalk ablation in *L. vannamei* (Kang et al. 2014). In another paper, we clarified the structure of multiple VIH genes present in *L. vannamei*, and elucidated their expression levels in the eyestalks in relation to molting and unilateral eyestalk ablation (Kang et al. 2018). The results of such research will be presented in detail during the presentation, along with discussion of other endocrine factors besides VIH that potentially control ovarian maturation in shrimp. The over-arching aim of this research is to provide useful technology to developing regions of the world where shrimp culture is an important industry.

## KEYWORDS

*Litopenaeus vannamei*, Shrimp, Vitellogenesis-inhibiting hormone (VIH), Vitellogenin (Vg)

## REFERENCES

- Kang, B.J., Okutsu, T., Tsutsui, N., Shinji, J., Bae, S.-H., and Wilder, M.N. (2014). Dynamics of vitellogenin and vitellogenesis-inhibiting hormone levels in adult and subadult whiteleg shrimp, *Litopenaeus vannamei*: relation to molting and eyestalk ablation. *Biology of Reproduction*, 90(1)12: 1–10.
- Kang, B.J., Sultana, Z., Zhang, G., Chen, H.-Y., and Wilder, M.N. (2018). Gene structure and expression analyses of multiple vitellogenesis-inhibiting hormones in the whiteleg shrimp *Litopenaeus vannamei*. *Fisheries Science*, 84: 649-662.

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## Development of new aquaculture and seed production technology for commercially-important shrimp species: Research at JIRCAS

Japan International Research Center for Agricultural Sciences

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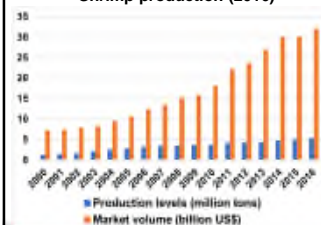
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## World-wide trends in shrimp culture

- Annual production levels: 5 million tons; market volume: 30 billion US dollars.
- Most significant species: whiteleg shrimp (*Litopenaeus vannamei*).
- World-wide industry: 75% occurs in South and Southeast Asia.
- More than half of all shrimp consumed world-wide is provided by aquaculture.

Shrimp production (2016)



Shrimp species (2016)

[FAO Statistics (<http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en>)]

## Background

### Major problems

#### 1) Adverse environmental impact

- Destruction of mangrove forests
- Self-pollution

#### 2) Disease outbreak

- White spot syndrome virus (WSSV)
- Early mortality syndrome (EMS)

#### 3) Difficult to induce female reproduction in captivity

- Unstable seed production

### Solutions

- Promotion of recirculating aquaculture systems (RAS)
- Use of specific pathogen-free (SPF) brooders and seed

- Elucidation of reproductive mechanisms
- Development of new techniques to control ovarian maturation in captivity

### Approaches at JIRCAS

- ◆ Development of environmentally-friendly, consumer-safe shrimp culture systems
- ◆ Development of an efficient, animal-friendly seed production technology

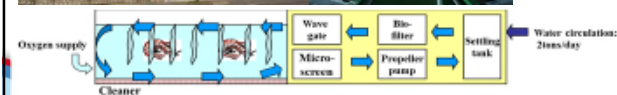


## ◆ Development of environmentally-friendly, consumer-safe shrimp culture systems

### ▶ The Indoor Shrimp Production System (ISPS) [FY2004 ~ 2008]

- ISPS No. 1 (Myoko City, Niigata Pref., Japan)
- Japan International Research Center for Agricultural Sciences (JIRCAS)
- IMT Engineering, Inc.
- National Research Institute of Aquaculture
- Higashimaru, Co., Ltd.

Japan's first commercial recirculating shrimp culture facility:



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## ◆ Development of an efficient, animal-friendly seed production technology

### Why we need new technology to promote ovarian maturation:

#### Requirements

- Approximately 200 billion post-larvae (juvenile shrimps) are produced world-wide annually to support the 5 million tons of *L. vannamei* that are produced annually.
- For example, 800,000 adult broodstock pairs are produced and exported from the state of Hawaii (From: Shrimpnews.com, Feb. 3, 2016; "Hawaii-SPF broodstock exports set record in 2015").

#### Artificial maturation

- How: Eyestalk ablation
- Why: Ovarian maturation and spawning are difficult to control in captivity; eyestalk ablation causes a higher proportion of shrimp to mature



## Conventional technology: eyestalk ablation

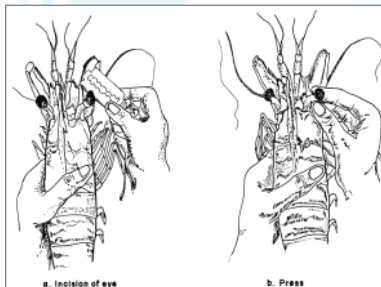


Figure referenced from: FAO Training Manual (1985) Shrimp Hatchery Design, Operation and Management

- Female broodstock normally produce up to 250,000 nauplii.
- Females subjected to eyestalk ablation can be used over several spawning cycles for 4 to 4.5 months.
- Fecundity drops gradually after several months and females are then discarded.

"Along with cutting and squeezing the prawn's eye, other methods including cauterisation (cutting the eyestalk with a heated blade or forceps) and ligation (tying a thread or wire around the eyestalk causing it to fall off after a few days)."

Quote from: Animals Australia for a kinder world (<https://www.animalsaustralia.org/features/prawn-farming.php>)



### Adverse effects of eyestalk ablation

From: **Animals Australia**  
for a kinder world  
(<https://www.animalsaustralia.org/features/prawn-farming.php>)

The shocking practice that shows prawn farming is as cruel as factory farming

**"The shocking practice that shows prawn farming is as cruel as factory farming"**

- Increased mortality rates
- Deterioration of egg quality
- Criticism from the viewpoint of animal welfare by the general public

New reproductive-stimulating technology is required to replace eyestalk ablation



### Outline of research project

#### JIRCAS Fourth Medium to Long-Term Plan (FY2016–2020)

#### Goal-Oriented Basic Research

- Basic research aimed to develop technologies that will lead to future innovation
- Novel technology development for the creation of new food industries

#### Project


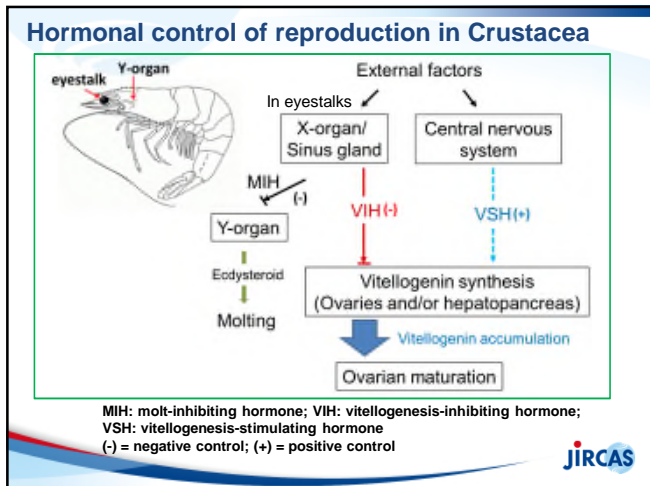
- Development of advanced seed production and culture technology for economically-important shrimp species based on elucidation of reproductive mechanisms

#### Purpose

- Development of new techniques to promote ovarian maturation and seed production in captivity

#### Approach

- Elucidate reproductive mechanisms in shrimp/Crustacea
- Develop technology based on the biological functioning of maturation-regulating factors

### Research results (1): Identification of VIH in *Litopenaeus vannamei*

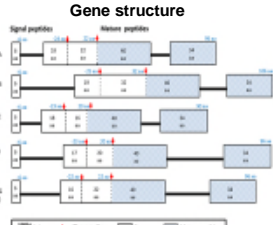
Subphylum: Crustacea  
Family: Penaeidae

- Most significant penaeid shrimp species farmed worldwide
- Frequently used research species in physiological studies

#### Sinus gland peptides (SGP) in *L. vannamei*

Name of peptide	VIH activity	Gene information
SGP-A	○	×
SGP-B	○	×
SGP-C	○	○
SGP-D	×	○
SGP-E	○	×
SGP-F	○	×
SGP-G	○	○

#### Gene structure



[Kang et al., 2018. Fisheries Science, 84: 649-662]

### Research results (2): Establishment of a measurement system for Vg and VIH levels in the hemolymph

#### Time-resolved fluoroimmunoassay for vitellogenin (Vg)

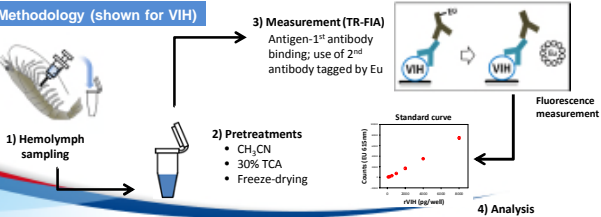
- Can quantify Vg from low volumes of hemolymph ( $\geq 0.1 \mu\text{L}$ ).
- Possible to assess maturity levels in female shrimp.

#### Time-resolved fluoroimmunoassay for vitellogenesis-inhibiting hormone (VIH)

- High sensitivity; lowest detectable quantity is about 9 pmole of VIH.
- Possible to examine the relationship of VIH levels with maturation.

#### Methodology (shown for VIH)

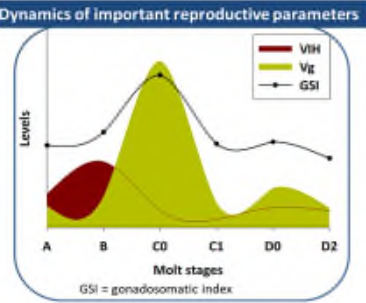
- Hemolymph sampling
- Pretreatments
  - $\text{CH}_3\text{CN}$
  - 30% TCA
  - Freeze-drying
- Measurement (TR-FIA)
  - Antigen- $1^{st}$  antibody binding; use of  $2^{nd}$  antibody tagged by Eu
- Analysis



### Research results (3): Dynamics of Vg and VIH in relation to molt stage


- In adults, Vg and VIH (SGP-G) levels fluctuate according to molting stage.
- Vg is induced after a surge of VIH (SGP-G) levels at molt stage B, and reaches a peak at molt stage C.

#### Dynamics of important reproductive parameters




GSI = gonadosomatic index

[Wilder, M.N., Kang, B.J., Higano, J. (2018). Vitellogenesis & Yolk Proteins, Crustaceans and Molluscs, In: Volume 6: Comparative Reproduction, The Encyclopedia of Reproduction. Elsevier, pp. 290-296.]



### On-going and future research

"A method of releasing the suppression of egg maturation in economically-important prawn/shrimp species"



Patent-pending

Suppresses the expression of the VIH gene, thus making it easier to induce ovarian maturation

**X-organ / sinus glands (eyestalk)**

~~(Vitellogenesis-inhibiting hormone: VIH)~~

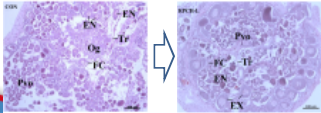
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**Ovary / Hepatopancreas**

**Removal of the influence of inhibitory factors**

✓ **Search for maturation-promoting factors**

- Candidate: red pigment-concentrating hormone (RPCH)



RPCH is:

- A peptide consisting of 9 amino acid residues.
- Expressed in multiple tissues including the eyestalks.
- Involved in the control of body color.
- Found to be involved in ovarian growth.

Increase in oocyte diameter after RPCH injection [Chen et al., 2018. Aquaculture, 495: 232-240]

### Conclusions


**This research has made possible the following:**

- ✓ Elucidation of the dynamics of Vg and VIH in the hemolymph according to molt stage and the related process of ovarian maturation.
- ✓ Elucidation of the expression levels of Vg and VIHs in relation to molt stage and the related process of ovarian maturation.
- ✓ Artificial suppression of VIH gene expression.

**Further work**

- Search for the putative vitellogenesis-stimulating hormone (VSH).
- Development of new methods for promoting ovarian maturation in captivity.

We hope to develop new technologies that can be used in hatcheries in major shrimp-producing countries in line with JIRCAS's mission of "To undertake comprehensive research in agriculture, forestry and fisheries, and assist related industries in developing regions".



## Thank you for your attention



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