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fight off challenges and  
increase market share  
in Western Europe**

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**SNAKEHEAD FISH**



**SEABASS**



**PANGASIUS**



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SHRIMP IMPROVEMENT SYSTEMS

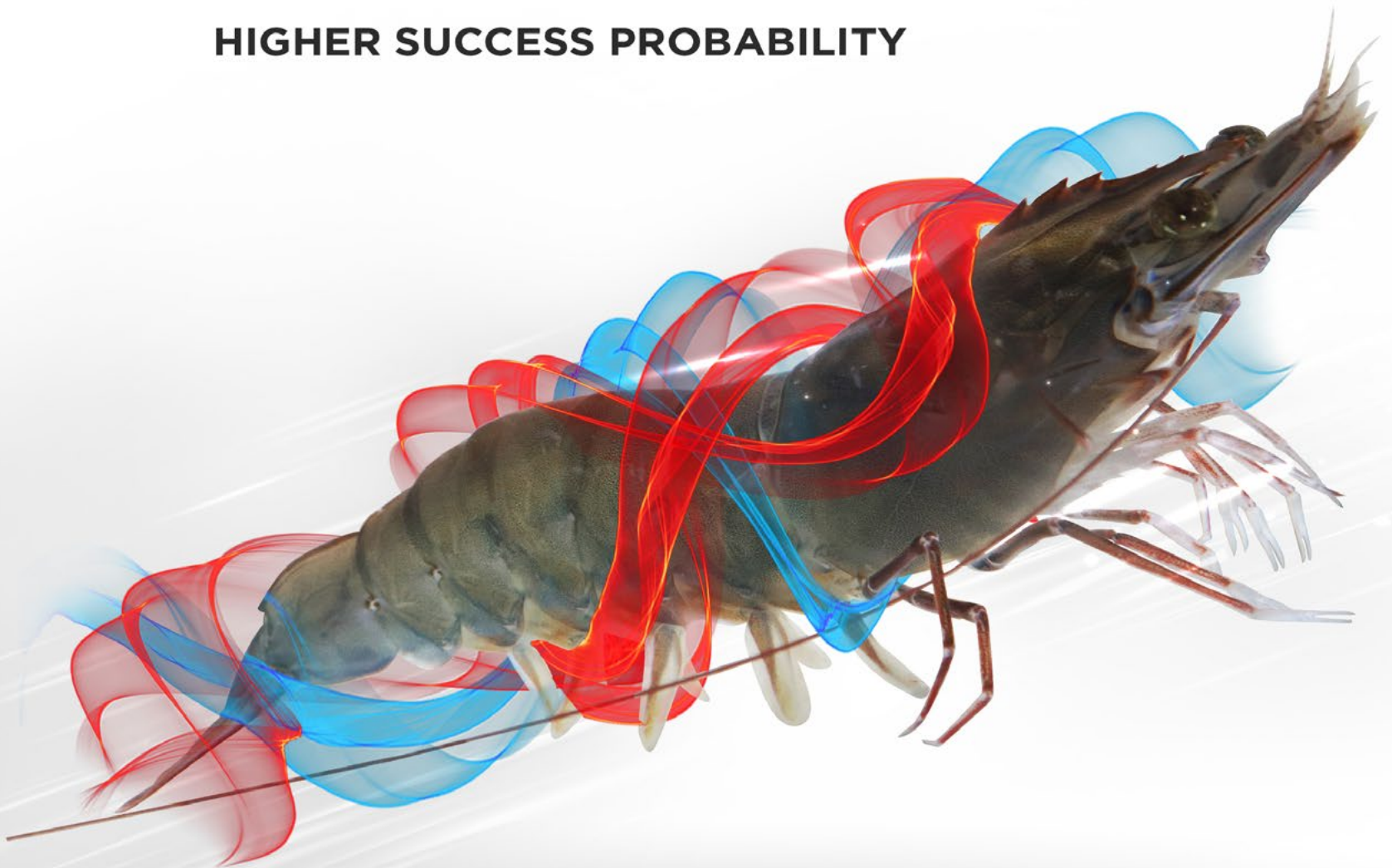
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## Shrimp exports to US exceed 1 billion USD for the first time

Exports of shrimp to the U.S. hit US\$1 billion in 2021, an increase of 20 percent compared to 2020, general secretary of the Vietnam Association of Seafood Exporters and Producers (VASEP) Truong Dinh Hoe highlighted at during a conference on shrimp industry development in 2022. The U.S. is currently Vietnam's largest shrimp market, accounting for 28 percent of total shrimp export value, according to the agricultural ministry. Shrimp exports to this country crossed the \$1 billion mark for the first time in 2021, up 20 percent from 2020, with a volume of nearly 90,000 metric tons. Experts predicted that the U.S. would remain the largest shrimp importer of Vietnam this year thanks to the rising demand of the food and hotel industries.

## The EU turn their back on white meat fish of Russia, creating opportunity for Vietnamese pangasius

Some supermarkets in Europe start to reduce their purchases of pollock and haddock from Russia despite an increasing demand for white fish in the EU countries. This is opening up a good opportunity for Vietnamese pangasius. In the EU market, Vietnamese pangasius has to compete against some kinds of white fish such as Russian pollock and haddock. Vietnamese pangasius is imposed an import tax rate of 5.5% while pollock enjoys a free duty policy. Nevertheless, in the next two or three years, the import tax rate imposed on Vietnamese pangasius will be 0%. The EU market crucially depends on Russia's white fish. Thus, it will be a big challenge for white meat fish importers into Europe to find substitute resources when seafood is prohibited in this market.

### BINH DINH

## Apply UFB technology into ocean tuna preservation

Ocean tuna accounts a large density of Binh Dinh's aquatic exploitation. The province has 3,200 offshore fishing vessels, nearly 1,500 of which are specialized in catching ocean tuna. In the first three months of 2022 alone, the output of wild-caught ocean tuna reached 3,265 tons, up 19.3% from the same period last year. In quarter II of 2022, the Sub-department of Fisheries worked with the Center of Agriculture Extension to select two qualified fishing vessels that will be instructed with techniques and transferred the UFB technology. The UFB technology helps fishermen preserve ocean tuna for a long time and keep the fish always fresh.

## Exports of tuna to Portugal skyrocket by 135%

After a growth of 75% in 2021, Vietnam continued to see a three-digit increase in exporting tuna to Portugal in the first three months of 2022. The export value reached USD 1.1 million, up 135% from the same period last year. In 2022, preferential tariffs upon EVFTA promise to facilitate Vietnamese tuna exported to Portugal. Moreover, the EU's policy of opening to tourism will result in an increase in demand for high value tuna products in this country. Thus, the EU, Portugal included, will increase their purchases, leaving a good opportunity for Vietnam's tuna enterprises to push up exports in the future.

## Try to achieve 1.4 million tons from marine production in 2030

In the 2022 plan of marine production, the country intends to spend 90 thousand hectares (polyculture excluded) in marine production and achieve 9.5 million cubic meters of cages. The country plans to harvest 790 thousand tons from marine production. According to the Directorate of Fisheries, the plan up to 2025 involves as many as 280 thousand hectares of land being used for marine production with a total 10 million cubic meters of cages and the estimated output of 850 thousand tons. By 2030, the marine production area will reach 300 thousand hectares with farming cages reaching 12 million cubic meters and output reaching 1.4 million tons. With the vision to 2045, Vietnam's marine production industry will reach an advanced level with a modern management method. Marine production will play an important role in the fisheries, contributing over 25% of the total output and achieving a worth of over USD 4 billion from exportation.

## Vietnam and India cooperate for fisheries development

India is one of the top ten leading trade partners of Vietnam and Vietnam is in the top 17 trade partners of India in the world and the top four biggest partners in the ASEAN region. In 2021, the bilateral relation brought in over USD 12 billion. India is hoping to work out a cooperation scheme for further trade development between the two countries. Seafood is one of potential products that Vietnam exports to India. In the first four months of 2022, Vietnam's seafood export value to India reached USD 7.2 million, up 22% from the same period last year, however, accounted for just a small amount of the total Vietnamese products going to India. Thus, Vietnam can increase its sales of seafood in this market.

## Innovation to improve the efficiency of the agricultural sector

On May 8, the International Conference on High-Efficiency Agriculture in Vietnam in 2022 was held in online form.

Speaking at the opening of the workshop, Minister of Agriculture and Rural Development Le Minh Hoan said that the Prime Minister has issued the Strategy for Sustainable Agriculture and Rural Development in the 2021 - 2030 period, with a vision to 2050. This is the first time. First, Vietnam's agriculture has a long-term strategy that integrates both agriculture and rural areas. Based on the content of the Strategy, the country's agricultural industry needs to harmonize and integrate the value of the 4.0 revolution with knowledge agriculture and smart agriculture. Accordingly, it is suggested that there should be a forum to connect experts to share information, and initiatives and develop action plans to solve the problems that Vietnam's agriculture still faces, and support the economy. Vietnamese agriculture moves faster and has access to more new technologies.

### VIETNAM - JAPAN

#### Technology innovation cooperation

Within the framework of the visit to Vietnam by Japanese Prime Minister Kishida Fumio, on May 1, the Ministry of Industry and Trade coordinated with the Ministry of Foreign Affairs, the Ministry of Information and Communications, the Ministry of Planning and Investment, and the Japan Trade Promotion Agency (JETRO) organized the Workshop "Vietnam - Japan cooperation in technological innovation, digital transformation and supply chain diversification". Information at the Conference showed that technological innovation, digital transformation, and diversity Supply chain form is a popular trend and a process of profound change in industrial production activities in many countries around the world, including Vietnam. More than ever, the need for technological innovation and digital transformation is becoming urgent for Vietnamese businesses to improve their competitiveness, and production efficiency and be ready to receive a new wave of investment in the future. efforts to diversify the supply chains of multinational corporations. However, this is not an easy process with numerous challenges, requiring appropriate and comprehensive solutions.

### VIETNAM - NETHERLAND

#### Enhance the efficiency of agricultural development cooperation.

The total fishery production in the first 4 months of 2022 of Ca Mau province reached over 200,000 tons. In which, fishing output reached 77,000 tons, aquaculture production reached nearly 130,000 tons. The aquaculture area accounts for 280,000 hectares, of which the super-intensive and intensive shrimp farming area is nearly 8,000 hectares. The improved extensive shrimp farming area is 172,000 ha, the rest is cultured in combination with other objects.

Information at the meeting and working session with Ms. Elsbeth Akkeman - Ambassador Extraordinary and Plenipotentiary of the Kingdom of the Netherlands in Vietnam on the afternoon of 4/5; Minister of Agriculture and Rural Development Le Minh Hoan said that up to now, the Netherlands is the EU's largest foreign investment partner (FDI) in Vietnam with nearly 400 projects and total capital of about 11 billion USD, accounting for nearly 50% EU's total investment capital in Vietnam. The Minister highly appreciated each relationship between Vietnam and the Netherlands, especially cooperation in the field of agriculture. Speaking at the visit and working session, Ms. Elsbeth Akkeman said that the Dutch Embassy in Vietnam is committed to accompanying and supporting businesses in the region.

VFM

■ Although the current price of raw shrimp is at a high level, farmers are excited, however, fluctuations in the market have made most of the raw materials and inputs increase sharply, causing the cost of farming to increase accordingly. Specifically, the price of shrimp feed from the beginning of the year to now has increased 2-3 times with a total increase of about 900 - 1,200 VND/kg; seed also increased by about 20 dong/head.

■ Tinh Bac Kan has 2 rivers, namely Nang and Cau rivers, and 35 irrigation reservoirs that can raise fish. Currently, many people in Ba Be, Ngan Son, and Cho Moi districts are exploiting this advantage, with the main farming subjects being: monogamous tilapia, red tilapia, grass carp, black carp, etc. The difficulty for farmers is the lack of capital, especially the cost of investing in fish cages.

■ In 2022, the Lang Son Agricultural Extension Center will deploy the model "Breeding American catfish in cages on rivers and in storage, associated with product consumption links". The model has a total cost of 782,640,000 VND to implement the scale of 800 m<sup>2</sup> cages, stocking at a density of 10 fish/m<sup>2</sup>. The cost of the meal is supported by the State 70% of the cost, the rest is the corresponding household.

■ Currently, in Quảng Ninh province, there are 18 establishments producing and rearing aquatic breeds, of which 4 are mainly producing freshwater seed, 14 are mainly producing and nursing brackish seed. Breeds of brackish saltwater fish meet more than 30% of the demand for farmers; Freshwater aquatic products meet over 95% of the demand.

■ The whole province of Nam Định currently has 16,000 hectares of aquaculture, mainly in the districts of Hai Hau, Giao Thuy, and Nghia Hung with many different farming forms such as ponds, lagoons, tanks, cages... In 2022, the province strives to expand the aquaculture area to 16,015 hectares with a total output of 127,500 tons.

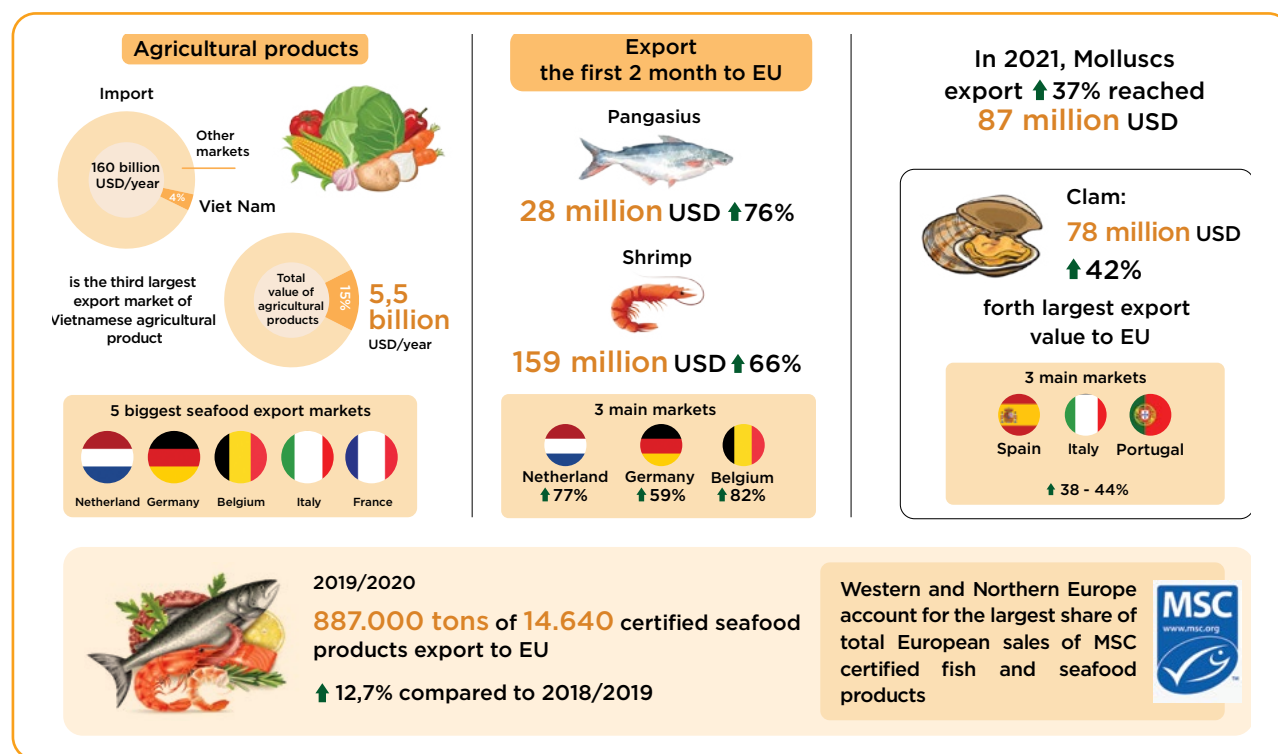
■ According to the Phu Yen Department of Agriculture and Rural Development, in the first quarter of 2022, the whole province exploited nearly 17,800 tons of aquatic products of all kinds, up 6.3% over the same period last year. Particularly, tuna fishing vessels increased by 20-30% with a total output of 1,217 tons, an increase of nearly 20%. At the same time, the price of tuna increased, to 145,000 - 150,000 VND/kg.

■ The total fishery production in the first 4 months of 2022 of Ca Mau province reached over 200,000 tons. In which, fishing output reached 77,000 tons, aquaculture production reached nearly 130,000 tons. The aquaculture area accounts for 280,000 hectares, of which the super-intensive and intensive shrimp farming area is nearly 8,000 hectares. The improved extensive shrimp farming area is 172,000 ha, the rest is cultured in combination with other objects. ■

## VIETNAMESE SEAFOOD

# Increase market share in EU

The EVFTA has paved a way for Vietnam to export agricultural products with competitive prices in the EU market. At the same time, the on-going tension between Russia and Ukraine will change history and create a new picture of politics and economy of the EU.



the Netherlands, and Belgium are the biggest importers. In the first quarter of 2022, only sales to Portugal slightly decreased by 9%; the other markets showed a double-digit growth, from 33 to 45%; Italy was the biggest single importer of Vietnamese clams with a worth of USD 6.4 million, up 45% from the same period last year. Clam exports to Spain reached USD 6.3 million, up 44%.

## Impress in the Seafood Expo Global 2022

The Seafood Expo Global 2022 was held in Barcelona city of Spain from April 26 to 28. Three Vietnamese enterprises came to introduce clam products: Godaco, Beseaco, and Aquatex. During the first two days of the Expo, the stalls of Vietnamese clam were visited by nearly 100 importers and distributors from Spain, Italy, France, the US, Portugal, the UK, Turkey, the Netherlands, UAE, Korea, Egypt, Israel, and Denmark.

After talking with consumers, Vietnamese enterprises realized the clear requirements and necessity of MSC certification. MSC is being paid the most attention in the world, helps prove sustainable fisheries, and is recognized as a passport for exported seafood products.

## Seafood supply shortage

The Russia-Ukraine tension made many EU consumers turn their back on Russian products. The retail system in the UK has boycotted seafood products from Russia. This may become a trend in other countries if the war is not over. Spain has banned the importation of Russian seafood and prohibited Russia vessels and boats from docking in harbors of Spain and other EU countries.

## Blooming exportation

Lê Hằng, Deputy Director of VASEP.PRO center, the EU is a big market with various segments and products, creating a favorable condition for Vietnam's exportation of seafood products. Besides, the European people have been increasingly in favor of white fish products for their health benefits; meanwhile the awareness of environmental and natural fish protection has been raised, leading to considerable opportunity for farmed fish, especially pangasius. Revenue from seafood exports has recovered after the Covid-19 pandemic and the EU is leaning towards convenient and instant food. Moreover, the EVFTA gives Vietnam an advantage related to import duties over other exporters, and the affection of Brexit against the EU market also offers opportunities for Vietnamese seafood.

The EU always demands abundant resources due to the average consumption of 24.35 kilogram of seafood per capita. While the strike between Russia and Ukraine is occurring in Europe, demand for storage and consumption of seafood increases.

According to the Ministry of Industry and Trade, in the first two months of 2022, pangasius exports to the EU reached USD 28 million, up nearly 76% and shrimp exports reached USD 159 million, up 66% as compared to the same period last year. Exports to the biggest markets, Netherlands, German, and Belgium increased by 77%, 59%, and 82% respectively.

Among advantageous products going to the EU market, clams are showing positive signs in sales. The EU has been the biggest importer of Vietnamese seafood, accounting for 70% of the country's

total exported products, trailing the US, Singapore, and Japan. As clam products are produced upon MSC standards for food safety assurance, they easily penetrate into supermarkets, high-end restaurants or demanding markets such as the EU and Japan. Packages with MSC label prove to consumers safety and environmental and social responsibilities.

The EU is in favor of products such as clam meat, steamed whole white/brown clams, clams steamed with butter and garlic, clams mixed with tomyum, etc. Vietnamese clams have been increasingly attractive to the European consumers amidst inflation and skyrocketing prices of food in this market. Thanks to preferential tariffs upon the EVFTA, Vietnam's exportation of clams to the EU is forecasted to grow considerably this year. Among the EU countries, Italy, Spain, Portugal,

**>> Nguyễn Quốc Toàn, Director of Agrottrade Vietnam:** Vietnam has an advantage of having a large amount of wild-caught and farmed species. While markets are struggling with short supply, we have enough for exportation, especially to the EU, the US, and Russia. This is the moment for enterprises to take advantage of the EVFTA and UKVFTA to boost exportation of all products. Seafood enterprises must satisfy requirements of quality control and seize opportunities to conquer the market, especially the segment of pollock (Russia).



White fish products from Russia have been boycotted and can not be paid by foreign currency, resulting in a higher demand for white fish in the EU. This is one of reasons for the remarkable recovery of Vietnam's sales of pangasius products with USD 646 million worth in the first three months of the year, up 88% from the same period of 2021.

The war in Ukraine led to an increase in prices of feed ingredients and charges of transportation. Prices of wheat and corn rose by 10-20%. In Vietnam, prices of pangasius material increased by 30-40%, making factories struggle in purchasing materials.

However, exports of Vietnamese products to the EU and Russia will continue to benefit from free trade agreements and long-term partnerships. Vietnam's enterprises and people are looking forward to a peace in the EU and sustainable exportation, accordingly, Vietnamese products will easily approach consumers of Russia, Ukraine and other EU countries.

**NGUYỄN ANH - DIỆU AN**



### **NGUYỄN THỊ HOÀNG THỦY, COMMERCIAL COUNSELOR IN SWITZERLAND AND DENMARK, FINLAND, ICELAND, NORWAY AND LATVIA** **Bright picture for pangasius**

Even though the average consumption is high in Northern Europe, Vietnam has exported just a small amount of seafood to this market. However, not to mention the EU member countries, Vietnam is the second biggest exporter to the EU, after China. Vietnam does not have any competitors when selling pangasius to Northern Europe, and yet the export volume is low due to the geographically remote and small-scale market. Northern European countries have to buy Vietnamese products from other markets such as the Netherlands, Germany, Belgium, and France. Clearly, this is an ideal opportunity for Vietnamese pangasius. Vietnamese shrimp is the key product going to Northern Europe, and Vietnam is the second-largest exporter of shrimp to the EU.



### **LÊ HOÀNG TÀI, DEPUTY DIRECTOR OF VIETNAM TRADE PROMOTION AGENCY UNDER THE MINISTRY OF INDUSTRY AND TRADE** **Achievements from EVFTA benefits**

The EU is one of the leading importers of Vietnamese seafood. On August 01, 2020, the officially effective EVFTA with preferential tariffs offered plenty of opportunities for Vietnamese seafood to enter the EU market. As a result, in February of 2022 alone, Vietnam achieved USD 28 million from selling pangasius to the EU, up nearly 76%, and USD 159 million from exporting shrimps, up 66% as compared to the same period of 2021. Exports to the Netherlands, Germany, and Belgium increased by 77%, 59% and 82% respectively. As the EU's demand for shrimp rises in summer and autumn, local importers usually prepare for orders at the beginning of the year.



### **NGUYỄN NHƯ TIỆP, DIRECTORATE OF NATIONAL AGRO-FORESTRY-FISHERIES QUALITY ASSURANCE DEPARTMENT** **Huge demand for mollusk in EU**

The world is always in high demand for bivalves such as clam, blood cockle, sweet snail, scallops, abalone, oyster, etc. In the EU market, bivalves are also the main seafood products to be consumed. Last year, exports of mollusks to the EU increased sharply by 37%, valued at USD 87 million, due to a noticeable increase in sales of clam by 42%, valued at USD 78 million. However, Vietnam's total output of clam can not fulfill orders from the EU because just a small amount of clam is certified for origin. Besides, there are not plenty of clam products, the value-added level is low, advertisement and trademark promotion is poor, and markets are not diversified. Moreover, some cargoes of bivalves going to foreign countries were given warnings, three of them destined for the EU received a warning for Salmonella infection in 2021.



### **TRƯƠNG ĐÌNH HÒA, GENERAL SECRETARY OF VASEP** **Must satisfy standards regulated by the EU market**

The EU has tightened the application of Good Manufacturing Practice in accordance with responsibilities for society and environment when it comes to exported seafood. Thus, Vietnamese enterprises must: label products exactly, obey compulsory regulations imposed by importing markets, satisfy additional regulations of single markets in the bloc, follow up the information of economy, politics, and COVID-19 pandemic so as to give prompt prediction and solutions, seek for opportunities to increase market share of pangasius amidst the short supply of white meat fish in the EU, control product quality well to keep credibility, take further advantage of the EVFTA in the future. Besides, enterprises should be given more specific instructions by competent authorities and supporting organizations.

**LINH NGUYỄN - HẢI LÝ**

# Vietnamese shrimp fight off challenges and increase market share in Western Europe

"If prices reduce, prohibited substances are well controlled, farming facilities are identified with codes, and ASC standard based farming models are multiplied, market share of Vietnamese shrimp in Western Europe will sharply grow in a sustainable way and gain the first position", Sao Ta Foods Joint Stock Company's Chairman of the Board Hồ Quốc Lực told VFM when asked about the advantages and challenges of Vietnamese shrimp in Western Europe.



**Would you give some evaluation about the Western European market for Vietnam's seafood exportation?**

After Brexit happened in 2020, we seemed to have made a mistake when targeting the European market and the UK market separately, because the UK has been the leading country in consuming food, seafood included. On the other hand, the regulations on quality control over imported products of these two markets are not quite different. Thus, when

giving an evaluation about the Western European market, the EU and UK should be included.

The UK's regulations of tariffs imposed on Vietnamese seafood are not quite different from the EU's. The big food distribution systems in Western Europe penetrate into many countries in the bloc, the UK included. Thus, it is easy to sell products in Western Europe due to similar models. Ecuador has been the biggest provider of shrimp to Western Europe, Vietnam has been the second. However, it is

not the same in each member country. Western Europe has been the second or the third biggest importer of Vietnamese shrimp for years. Among the member countries, the UK, the Netherlands, Germany, and Belgium have been the most popular destinations of Vietnamese shrimp, mainly steamed shrimp, IQF fresh shrimp, packaged tempura shrimp sold in supermarkets, and breaded shrimp sold in supermarkets and restaurants. Besides, Cà Mau organic tiger shrimp are

popular and sold at high prices. Vietnamese shrimp have taken up good positions in high level distribution systems thanks to processing technologies and ASC certification which has helped gain trust from customers.

**In your opinion, what is the most advantage that Vietnamese shrimp have in this market?**

Vietnamese shrimp have lots of advantages in the Western European market. First of all, it is the EVFTA (2020), upon which import taxes would reduce as



scheduled, even taxes imposed on HS03061792 and HS03061799 have reduced to zero, increasing the competitive advantage of Vietnamese shrimp. The second is Vietnam's high level skills in processing shrimp which have enabled Vietnamese shrimp to enter high level distribution systems. Accordingly, prices will be better.

However, as compared to the competitor Ecuador with its white leg shrimp, Vietnam is setting a higher price for the same product. This is attributed to lower survival rate, higher production costs, and less professional farming process as compared to Ecuador. However, Ecuador is only good at selling fresh shrimp, thus, Vietnam has an opportunity for processed shrimp. In the next few years, if Ecuador updates its processing technology, and yet Vietnam can not lower the production cost of shrimp farming, Vietnamese shrimp will face more serious difficulties than they do now. Bangladesh is another competitor with cheaper black tiger shrimp, however, the models of processed shrimp are simple, leaving no big worries.

#### **What is the biggest challenge faced by Vietnamese shrimp in this highly potential market?**

Prices do not challenge Vietnamese shrimp, but food safety, traceability and the way to increase market share to the high-end market.

Western Europe is a demanding market with stringent food safety control regulations. After clearance, products may be rechecked by the quality control division of the distribution channel. Meanwhile, Vietnamese shrimp, which are reared by small

households and are not controlled by competent authorities, may face lots of risks. Moreover, traceability is a must, not only in Western Europe but also in all markets. Consumers require traceability, not only in grow-out ponds, but also in supply chains (post-larvae, feed, etc.); meanwhile the identification of Vietnamese shrimp farming facilities is stagnant. This matter must be solved if Vietnam's enterprises want to prove to consumers their ability in controlling materials. As for market share increasing, only ASC standard based shrimp farming areas are able to enter into the high-end segment; however Vietnam has a few farming areas certified with ASC standard. Small farming areas and high costs are to blame.

#### **Would you suggest some solutions to increase the ASC farming areas?**

In my opinion, there should be more projects to call for investors into shrimp farms and policies of saving lands to build hundreds-hectare shrimp farms. Only large-scale farms can be equipped to fully carry out the intensive farming model, reduce risks, increase productivity, push down production costs, and enhance the competitiveness of Vietnamese shrimp. When Vietnam obtains hundreds of hectares of ASC shrimp farms, Vietnamese shrimp will surely conquer the high-end segment of the Western European market.

#### **Is it possible that Western Europe is still a potential market of Vietnamese shrimp?**

Absolutely. In my opinion, Western Europe is still a huge and potential market. However, we

## **The trend of consumption of seafood in EU**

The EU's consumption index of seafood has been higher than meat for years due to consumers' leaning towards seafood. In the EU market, retail supermarkets are the main distribution channel, accounting for the largest market share of the distribution system of seafood products. The EU consumers attach importance to labels, packages, quality, etc. Around 70% of seafood is consumed at home, the rest is served at restaurants. The COVID-19 pandemic changed the consumption habit and importation of seafood in the EU market. Consumers like to buy seafood products that are averagely cheap, convenient and easy to preserve or cook at home.

Vietnam's seafood products that have advantages in EU:

- Frozen, canned, dried, and processed seafood such as fish ball (which is mainly consumed by Asian European people).
- Vietnam's frozen pangasius is competitive due to reasonable prices and satisfactory processing techniques.
- White leg shrimp and frozen small or medium size black tiger shrimp are favored in the EU.

## **Some regulations and specifications of exportation to the EU**

*Acceptable countries and companies:* based on the similarity between community health and control systems. When an exporting country is accepted, its competent authorities have the right to approve companies. The approved companies are granted unique identification numbers.

*IUU regulation:* EC's regulation no.1005/2008 dated September 29, 2008 regarding the establishment of prevention and removal of IUU fishing activities.

*Health certificate:* Seafood products exported to the EU must be granted health certificates by exporting countries' competent authorities provided that their health-care and disease control systems are equal to the EU's; also cargo transported to the EU must follow the EU's regulations.

*Food safety:* food safety is an important content in the Law of European Food adjusted by the General Food Law. The EC regulation no.178/2002 dated January 28, 2002 refers to General regulations of food safety over all phases of production and distribution. The EC regulation no.852/2004 dated April 29, 2004 by the Parliament and European Council refers to food safety. Food safety is carried out based on management systems such as: ISO 9001, ISO 22000 and Hazard Analysis and Critical Control Point System (HACCP). HACCP is the first step towards more stringent food safety programs such as FSSC22000 or BRC. The Directive no.91/493/EEC dated July 22, 1991 refers to fish and fish products.

should notice that even though Vietnamese shrimp have certain advantages, the increasing rate of market share may not be as expected when challenges are not promptly dealt with. If production costs reduce, the residue of prohibited substances are well controlled, the identification of farming facilities

is fully performed, and the ASC shrimp farms increase, Vietnamese shrimp will increase market shares and obtain the leading position in this hard market.

*Thank you.*



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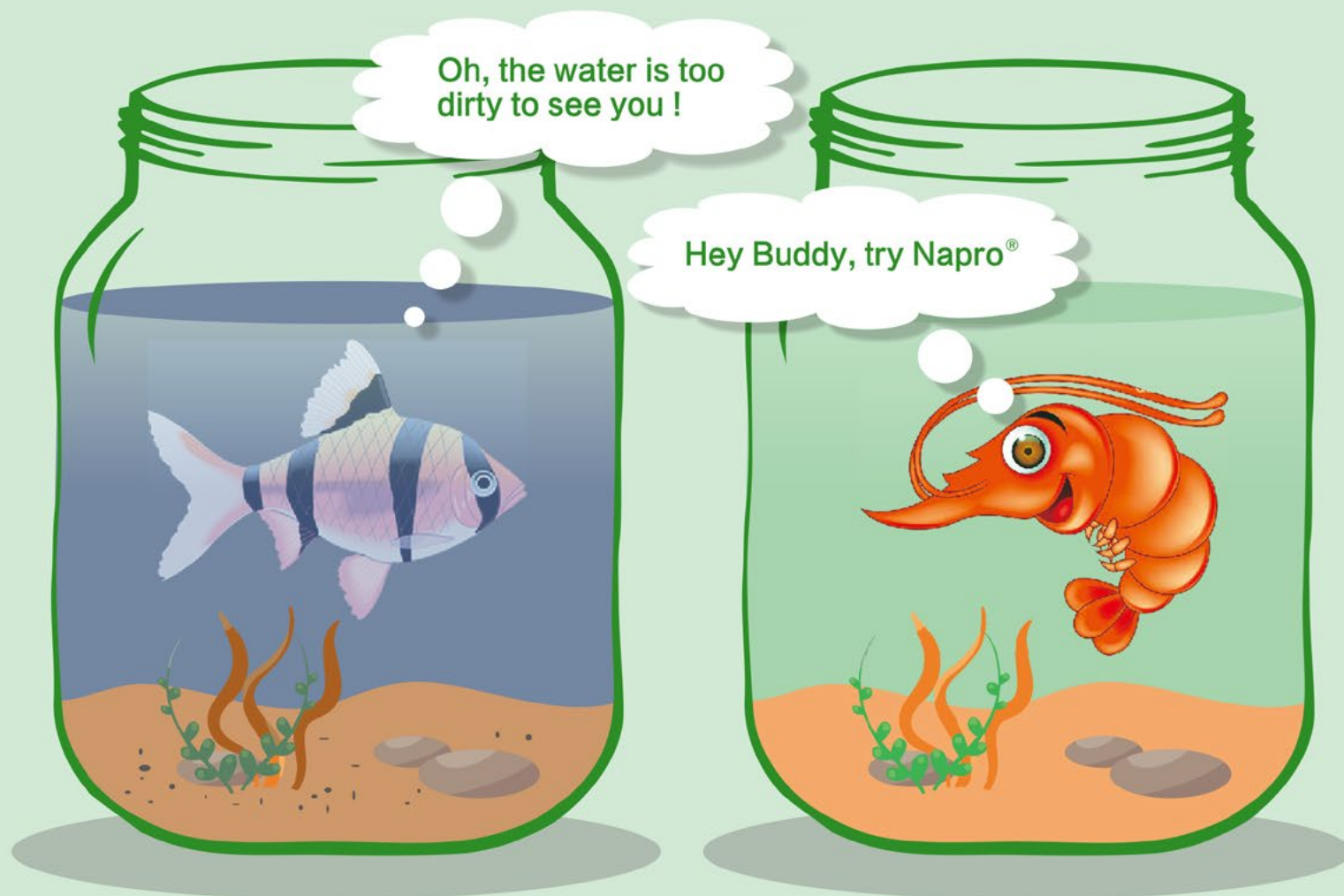


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## MINERAL SUPPLEMENTATION IN LOW SALINE CULTURE OF PACIFIC WHITE SHRIMP

## Effects on growth and water quality

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## 1. INTRODUCTION

*Penaeus vannamei* (pacific white shrimp) is a euryhaline crustacean. Being tolerant to salinities ranging from 0.5 PSU to 40 PSU, it is cultured in low saline water in many countries, mostly in inland ponds. The salinity and the ionic composition of inland ponds vary with the source of water. Even at favourable salinity levels, an imbalance of major minerals directly affects the growth and survival of shrimp (Chitra et al., 2017). Deficiency in the essential macro minerals like calcium, magnesium, potassium and sodium may limit the growth and survival of shrimp as each mineral is responsible for certain biological and physiological functions. Ca plays a role in exoskeleton formation especially after moulting and also acts as a cofactor for the enzymatic process along with osmoregulation; Mg is essential for cellular respiration and metabolic activity; K aids in cellular uptake of amino acids and Na is responsible for osmotic balance, enzymatic activity and osmoregulation. Generally, the deficiencies caused by insufficient minerals are remediated by either adding minerals to the culture water to modify its ionic composition or by supplementing minerals through the diet (Chitra et al., 2017; Davis et al., 2004). As crustaceans absorb most of the minerals from water through their gills and skin, application of minerals in water has been found effective. However, the concentration and the proportion of the minerals should be optimized as that of seawater to achieve optimum performance at any given salinity. Balanced composition of minerals in water helps in reducing the energy spent by animals to maintain the osmotic equilibrium (Chitra et al., 2017).

Studies have been carried out on the relationship between the proportion of major minerals in water and survival rate of *P. vannamei*. Aruna and Felix (2017) stated the need for maintaining the magnesium-calcium ratio at 3:1 for better survival and growth rate of *P. vannamei* in low saline waters, <9 PSU (Chitra et al., 2017). Roy et al. (2010) demonstrated that

## Abstract

As low saline inland shrimp culture is becoming more prevalent day by day, the application of mineral supplements in pond water has become a necessity to maintain the ionic concentration by fortifying the deficiency of key minerals like calcium (Ca), magnesium (Mg) and potassium (K). In this study, a 120-day trial was conducted in earthen ponds, wherein, the benefits of applying mineral supplement in low saline (>5 Practical Salinity Unit, PSU) was evaluated by measuring the animal performance parameters like feed conversion ratio (FCR), specific growth rate (SGR), survival rate, weight gain, mineral composition of experimental animals and the quality of rearing water like pH, dissolved oxygen (DO), salinity, hardness, alkalinity, biological oxygen demand (BOD), chemical oxygen demand (COD), *Vibrio* count and phytoplankton population in comparison with control. Our study showed that the application of mineral mixture improved the animal performance in terms of FCR, SGR and body weight. Despite, on supplementation, an increased mineral (Ca, Mg and K) content and improved phytoplankton levels was observed in rearing water whilst corresponding increase in minerals Ca, Mg and K was observed in animals as well. Indicating the improvement in water quality, mineral composition in water and animals and growth performance, affirming good absorption of minerals by animals.

## Keywords

Animal growth performance, mineral composition in animals, mineral deficiency, water quality

low saline waters, <10 PSU are generally deficient in K, Mg and sulphate, which significantly affect survival and growth of

*P. vannamei* and the increase in K and Mg would result in better growth. Davis et al. (2004) have studied and demonstrated the importance of Ca, K and Mg in the survival rate of *P. vannamei*. Roy et al. (2007) have also stated the need for maintaining Na:K at the ratio of 28:1 for the better survival and the growth of *P. vannamei*. Crucial roles of Na, Mg, K in growth rate and Na/K ratio for the survival of the shrimp have been reported (Bananmbang et al. 2019). Also, of importance is the form in which the mineral is supplemented. The organic forms of minerals have a greater bioavailability and absorption (Banrie, 2013).

This study was conducted to evaluate the effects of applying a mineral supplement in low saline culture system of pacific white shrimp – *Penaeus vannamei* on growth and pond water quality for a period of 120 days.

## 2. Materials and methods

## 2.1 Materials

Mineral mixture MasterMIN™ Aqua (M1) selected for the study is a commercially available product especially recommended to use in low saline cultures. The product is manufactured by Kemin Industries South Asia Pvt Ltd, India, which contains Ca (min. 4%), Mg (min. 4%), P (min. 1.5%), Zinc (min. 0.5%) and Mn (min. 800 ppm). The postlarvae (PL15) of *P. vannamei* used in this trial was procured from Jay Jay Gold Hatcheries, Pondicherry.

## 2.2 In vivo trial

A 120-day trial was conducted at Kerala University of Fisheries and Ocean Studies (KUFOS), Cochin, India. Six earthen ponds (4 ponds of 100 m<sup>2</sup> and 2 of 250 m<sup>2</sup> size) were equally divided into two groups - control and treatment, each group having three replicates.

To the treatment ponds, M1 was applied at 10 kg/acre dose, once every 15 days. The product was broadcasted to the ponds by hand. The control ponds did not receive any mineral supplementation.

### 2.3 Pond preparation and stocking

The ponds were filled with clean water from nearby lake, pumped through a mesh (500 microns) and filled up to the level of 1.5 m. Chlorination and dechlorination of ponds were performed. The ponds were made biologically active by applying a probiotic mixture prior to stocking prepared by mixing 6 kg each of jaggery, rice bran and groundnut oil cake in 200 L of pond water stored in a tank and fermented with 250 g of yeast for a period of 2 days with periodic stirring. The mixture was further applied at 7-day intervals after the ponds were stocked with specific pathogen-free (SPF) healthy PL15; average body weight 0.05 g of *P. vannamei*, at a stocking density of 60/m<sup>2</sup>. Each pond was provided with an aerator to counteract oxygen depletion and stabilize diurnal dissolved oxygen level. 20% of water exchange was performed twice during the study period to reduce ammonia levels but no periodic compensatory addition of water was performed. All the ponds were fed with PL feed for 3 days with four rations per day at the dosage of 2.5 kg per 100,000 PL. From the 4th day of stocking, a commercial starter feed (Marigold shrimp feed from Growel feeds, India) with a minimum level of 36% crude protein was given at the same rate with a daily increment of 5%.

### 2.4 Growth performance

Body weight, feed consumption, FCR and SGR were measured every 15 days from day 30 of culture by sampling 15–20 animals using check-trays set up at three different locations of each pond. On day 120, carapace width was measured by sampling four animals from each pond. The shrimp was harvested after 120 DOC and total bio-mass and survival percentage were calculated. The following equations were used for the calculations,

Average body weight(g) = Total body weight (g)/Total number of shrimps

Specific growth rate (SGR) = [Log final weight(g)-Log Initial weight(g)]/Number of days × 100

Feed conversion ratio (FCR) = Feed given (kg)/Body weight (kg)

Survival rate (%) = 100 × (Final number of shrimps- Initial number of shrimps)/Final number of shrimps

### 2.5 Mineral analysis

Content of Ca, Mg and K in shrimp muscle and culture water were analysed every 15 days by examining three animals and three water samples

from each pond. Determination of Ca, Mg and P content was performed by filtering, digesting and analysing using AOAC. 2011.14 methods by ICP-MS.

### 2.6 Water quality

Water samples were collected from three different locations of each pond in a plastic container and were analysed for the following parameters – pH, salinity, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), alkalinity, hardness, *Vibrio* count, calcium (Ca), magnesium (Mg), potassium (K) and phytoplankton count. Analyses were carried out before beginning the treatment and for every 15-day intervals during the treatment. pH, salinity and alkalinity were measured using pH meter, refractometer and APHA method, 1998 respectively. Dissolved oxygen was measured using Winkler method as discussed in the lab manual (Environmental Chemistry of Boston Harbor – IAP, 2006) whilst ammonia, nitrite and nitrate were measured using Merck water test kit. Total hardness was measured using EDTA titrimetric method as discussed in the lab manual (Standard Methods for the Examination of Water and Wastewater. Hardness by EDTA titration method [Lab manual], n.d.). To enumerate the total *Vibrio* count, the water samples were placed in a sample carrying box with ice packs and transferred to the lab. The collected water samples were analysed using plate count method in TCBS (Thiosulfate citrate bile salts sucrose) agar with reference to the APHA method. Phytoplankton counting was performed using microscopy method (Eva Willen, 1976). Ca, Mg and P were analysed using AOAC. 2011.14 method by ICP-MS.

### 2.7 Statistical analysis

Statistical analysis was performed using Stat Graphics Centurion XVI software (version 16.2.04). Data were analysed by One-way ANOVA and Multifactor ANOVA. *p*-value of <0.05 was considered statistically significant. Difference was measured between the groups on day to day basis.

## 3. Results

### 3.1 Growth performance

From day 45 onwards, the average body weight of M1-supplemented group was significantly higher (*p* < 0.05) with an increment of 5 g on day 120 compared to control (Figure 1). M1 group showed significantly lower FCR compared to the control group, throughout the trial (Figure 2). The final FCR values were 1.13 for M1 group and 1.56 for control. The growth performance of the experimental animals is shown in the Table 1. On completion of the trial period, the average body weight was found to be 36.27 g for

M1 group whilst animals in control ponds had average body weight of 31.21 g. The weight gain values recorded to be 31.16 g and 36.22 g with control and M1 added groups respectively. M1-supplemented group was found to have the SGR of 30.18% whilst 25.97% was observed in control group. The carapace width was 0.815 mm for M1 and 0.60 mm for control showing that animals of M1 group had a wider carapace. The survival rate of M1-supplemented group was 7.7% higher than the control group.

### 3.2 Mineral analysis

The comparison of mineral (Ca, Mg and K) content in shrimp on 15-day intervals from day 30 onwards (Figure 3a–c). Though the levels decreased as the trial progressed, significantly higher (*p* < 0.05) Ca content was observed in M1 group compared to control. The Mg content in M1 group decreased gradually until day 90 and increased on day 105 and day 120. Despite this variation, significantly higher (*p* < 0.05) level of Mg content was found in M1 group compared to C group. M1-supplemented group had significantly higher (*p* < 0.05) content of K compared to the C throughout the trial period.

### 3.3 Water quality

The general physico-chemical parameters of water measured during the study period is shown in Tables 2 and 3. The pH was found to be within the range (8.0–8.5) in all the ponds. The overall salinity of the water varied from 0.5 PSU to 4 PSU throughout the study period. A sudden dip in the salinity was observed on days 60 and 75, which could be due to heavy rains during those days. The salinity was found to gradually increase from day 90 with a significant difference showing higher salinity in M1 group compared to control. M1 group had significantly higher (*p* < 0.05) alkalinity on days 45, 75, 90, 105 and 120. Ammonia, nitrite and nitrate were found to be less than 0.1 mg/L in all the ponds throughout the trial period. BOD, COD and DO levels remained within the stipulated range in all the ponds. DO in M1 group was observed to be significantly higher on days 30, 60 and 75 than C group. Dissolved oxygen values were well above the minimum requirement

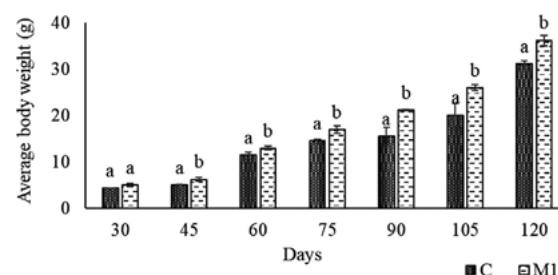


FIGURE1. Average body weight during the study period (Mean ± SD, n = 3). Statistical difference measured between the groups on day basis, different superscripts on the same day differ significantly (*p* < 0.05). M1 (MasterMIN™ Aqua) supplemented group showing significantly higher weight compared to control

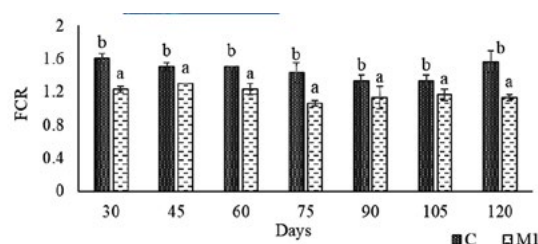


FIGURE 2. FCR during the study period (Mean  $\pm$  SD,  $n = 3$ ). Statistical difference measured between the groups on day basis, different superscripts on the same day differ significantly ( $p < 0.05$ ). M1 (MasterMIN™ Aqua) group showing significantly lower FCR compared to the control group

Table 1. Growth performance of *P. vannamei* at the end of study period

| Parameters              | C <sup>a</sup> | M1 <sup>b</sup> |
|-------------------------|----------------|-----------------|
| Average body weight (g) | 31.21          | 36.27           |
| Weight gain (g)         | 31.16          | 36.22           |
| SGR (%)                 | 25.97          | 30.18           |
| FCR                     | 1.56           | 1.13            |
| Carapace width (mm)     | 0.63           | 0.82            |
| Survival rate (%)       | 63.3           | 71              |

<sup>a</sup>Control.

<sup>b</sup>MasterMIN™ Aqua.

of 3.5 ppm. A significant decrease ( $p < 0.05$ ) was observed in BOD on days 45, 60, 75, 105 and 120, in the M1 group, which could be due to the higher phytoplankton population. No significant differences observed in COD between the control and treatment groups. M1 ponds had significantly lower ( $p < 0.05$ ) vibrio count, compared to C group except on day 0, 105 and 120.

The concentration of calcium, magnesium, potassium and hardness in three ponds (C & M1) is given (Figure 4a–d). A significant difference ( $p < 0.05$ ) was observed in Ca content from day 30, indicating application of M1 increased the Ca levels in water. There was a general decrease in the Mg content amongst the groups. At the end of the trial, M1 group had significantly higher ( $p < 0.05$ ) Mg content than C (Figure 4b). A significant difference was observed in K with M1 group showing higher content than C group except on day 0 and 90. The total hardness was observed to be significantly higher in M1 group than C, which could be due to the increase in overall mineral content. Also, higher phytoplankton population was observed in M1 group from day 15 until day 90 (Figure 4e).

#### 4. Discussion

In aquatic animals like shrimp, minerals are very essential and critical for basic growth and metabolism. For instance, Ca is essential for healthy exoskeleton, balanced tissue structure, membrane permeability, muscle contraction, nerve transmission and as a cofactor for enzymatic process. Magnesium is required by crustaceans for normal growth and development, osmoregulation and protein synthesis. Generally,

these major minerals are lost and become deficient due to utilization by the biotic community, soil adsorption, shrimp harvest, draining at harvest and seepage during the culture period (Pisal et al., 2006). The dietary requirement of minerals is largely dependent on the concentration of minerals in the culture water, as they can absorb minerals directly through gills and body surfaces. Increasing the bioavailability of the minerals in culture water can have a direct impact on the absorption by the animals (Aruna & Felix, 2017).

The present study examined the effectiveness of supplementation of mineral mixture in low saline shrimp culture water and its benefits on the animal growth and mineral composition of pond water. In the treated ponds, the general physico-chemical parameters of culture water were found to be in conducive range in all the ponds throughout the culture period. As a result of mineral supplementation, availability of minerals like Ca, K and Mg in culture water increased with the increase in salinity for all the ponds, which is also reflected in the hardness. This could be because of the higher solubility of the product in water leading to the corresponding increment of the minerals in the medium. Solubility of a mineral acts as a key indicator of the bioavailability of the same. Studies say that, when the salinity is increased, the ionic strength of the saline solution along with the solubility of the mineral mixture gets improved, which leads to the increased availability of various minerals (Bananmbang et al., 2019). But the uniformity in the increase in minerals was not observed because of the dynamic interaction of the minerals between soil and water. It was observed that the minerals Ca, Mg and K content in the animals were also found to increase with M1 application. This is because, mostly the aquatic animals absorb minerals from the environment, but it is highly selective.

The mineral concentration in an aquatic organism is largely dependent on the food source, environment, species type, developmental stage and physiological condition of the animal. Several factors like level, particle size, form and digestibility influence the bioavailability (Roy et al., 2007). Also, a significant improvement in performance parameters - average body weight, FCR, SGR as well as increased % survival was observed with the use of mineral mixture M1. This could be the result of increased mineral concentration observed in the pond water of M1. Many researchers like Roy et al. (2010) have demonstrated a positive correlation between survival and the concentrations of potassium, magnesium and sulphate in the water<sup>14</sup>. The studies by Aruna and Felix (2017) and Roy et al. (2007) revealed that the survival and production of *P. vannamei* is high at 3:1 of Mg:Ca ratio. Due to the increased availability of minerals, the phytoplankton population in

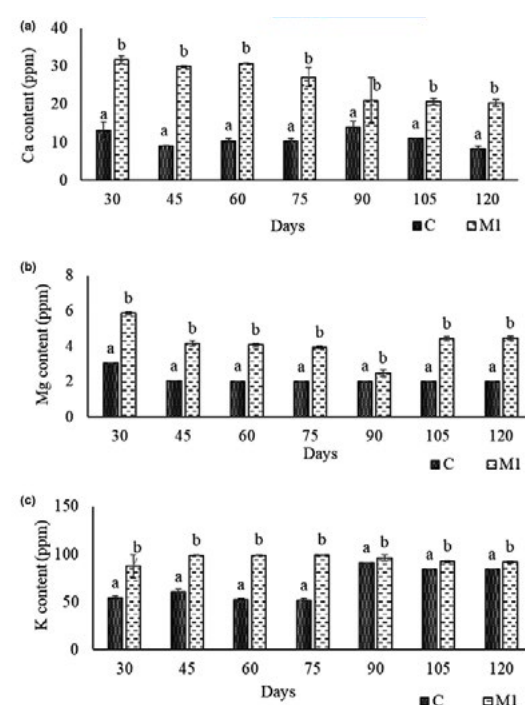


FIGURE 3. Mineral content in *P. vannamei* was measured in regular intervals during the study period. Statistical difference measured between the groups on day basis (Mean  $\pm$  SD,  $n = 3$ ), different superscripts on the same day differ significantly ( $p < 0.05$ ). The concentration of Ca, Mg and K are shown (a) significantly higher Ca content in M1 (MasterMIN™ Aqua) group compared to control, (b) significantly higher Mg in M1 compared to control and (c) M1 supplemented group had significantly higher ( $p < 0.05$ ) content of K compared to the C throughout the trial period

water was also observed to be high in M1 group than the control group, as nutrient-rich water results in high plankton population (Chanda et al., 2015). Studies by Hossain et al. (2007) have proved that optimal water quality and alkalinity have a strong positive influence on the growth of phytoplankton (Margalef, 1964). Increase in the phytoplankton must have contributed to higher population zooplankton, which might have been the reason for higher body weight of animals in the M1 groups as they serve as natural food. According to Bojorquez-Mascareno and Soto-Jimenez (2013), an adequate combination of formulated feed and natural food in ponds enhance the growth and development of shrimps (Roy et al., 2007). Reduction in the BOD was also observed in M1 group. BOD being the amount of oxygen required for the microbial metabolism of organic compounds in water. It is possible with a healthy phytoplankton population, which will enhance the oxygen level in water providing more oxygen for microbial breakdown of organic matter for their growth. Since this study was conducted during the monsoon period wherein complicated interactions between the weather, water and soil occurs some variations observed during the trial could not be properly understood nor explained within the scope of this study.

In conclusion, the study results clearly suggest that the application of mineral mixture (M1) leads to higher and better bioavailability of minerals in the culture medium, which is evident

TABLE 2. Average values of pH, salinity and alkalinity of pond water during the study period

| Parameters       | Days            | 0                           | 15                          | 30                          | 45                          | 60                          | 75                          | 90                          | 105                         | 120                         |
|------------------|-----------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| pH               | C <sup>§</sup>  | 8.43 <sup>a</sup><br>± 0.04 | 8.23 <sup>a</sup><br>± 0.03 | 8.06 <sup>a</sup><br>± 0.17 | 8.47 <sup>a</sup><br>± 0.02 | 8.50 <sup>b</sup><br>± 0.00 | 8.47 <sup>a</sup><br>± 0.03 | 8.50 <sup>a</sup><br>± 0.00 | 8.50 <sup>a</sup><br>± 0.00 | 8.50 <sup>a</sup><br>± 0.00 |
|                  | M1 <sup>§</sup> | 8.36 <sup>a</sup><br>± 0.11 | 8.17 <sup>a</sup><br>± 0.05 | 8.18 <sup>a</sup><br>± 0.02 | 8.46 <sup>a</sup><br>± 0.01 | 8.25 <sup>a</sup><br>± 0.14 | 8.39 <sup>a</sup><br>± 0.24 | 8.50 <sup>a</sup><br>± 0.00 | 8.50 <sup>a</sup><br>± 0.00 | 8.50 <sup>a</sup><br>± 0.00 |
| Salinity (PSU)   | C <sup>§</sup>  | 2.11 <sup>a</sup><br>± 0.11 | 2.67 <sup>a</sup><br>± 0.00 | 3.00 <sup>a</sup><br>± 0.00 | 4.00 <sup>a</sup><br>± 0.00 | 1.67 <sup>b</sup><br>± 0.33 | 0.67 <sup>b</sup><br>± 0.33 | 2.00 <sup>a</sup><br>± 0.00 | 2.67 <sup>a</sup><br>± 0.67 | 2.67 <sup>a</sup><br>± 0.67 |
|                  | M1 <sup>§</sup> | 2.08 <sup>a</sup><br>± 0.08 | 2.67 <sup>a</sup><br>± 0.00 | 3.00 <sup>a</sup><br>± 0.00 | 4.00 <sup>a</sup><br>± 0.00 | 1.00 <sup>a</sup><br>± 0.00 | 0.50 <sup>a</sup><br>± 0.29 | 2.00 <sup>a</sup><br>± 0.00 | 4.00 <sup>a</sup><br>± 0.00 | 4.00 <sup>a</sup><br>± 0.00 |
| Alkalinity (ppm) | C <sup>§</sup>  | 177.8 <sup>a</sup> ± 0.97   | 179.9 <sup>a</sup> ± 0.73   | 174.0 <sup>a</sup> ± 5.17   | 177.5 <sup>a</sup> ± 0.08   | 171.7 <sup>a</sup> ± 1.67   | 117.6 <sup>a</sup> ± 16.22  | 106.9 <sup>a</sup> ± 6.70   | 106.7 <sup>a</sup> ± 6.67   | 106.7 <sup>a</sup> ± 6.67   |
|                  | M1 <sup>§</sup> | 179.1 <sup>a</sup> ± 2.01   | 180.3 <sup>a</sup> ± 1.19   | 182.8 <sup>a</sup> ± 2.22   | 198.4 <sup>b</sup> ± 0.58   | 163.6 <sup>a</sup> ± 11.10  | 165.4 <sup>b</sup> ± 11.92  | 139.0 <sup>b</sup> ± 9.61   | 150 <sup>b</sup> ± 1.08     | 149.3 <sup>b</sup> ± 0.67   |

§Control.

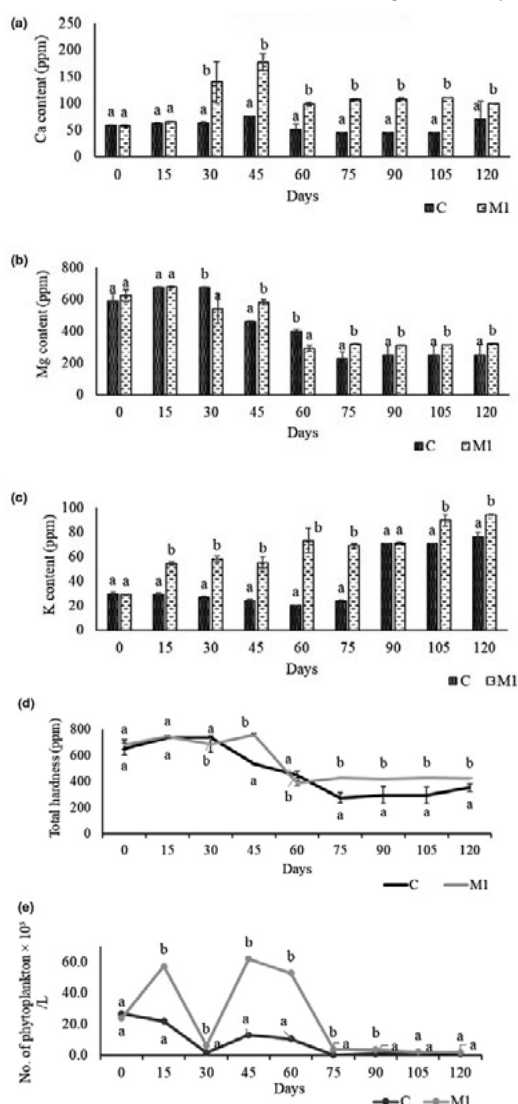
¶MasterMIN™ Aqua. All values are mean ± SD (n = 3). Values with different superscripts in a column of each parameter differ significantly (p < 0.05).

TABLE 3. Average value of DO, BOD, COD and Vibrio count of pond water during the study period

| Parameters                            | Days            | 0                           | 15                          | 30                          | 45                          | 60                          | 75                          | 90                          | 105                         | 120                         |
|---------------------------------------|-----------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| DO (ppm)                              | C <sup>§</sup>  | 4.22 <sup>a</sup><br>± 0.04 | 4.10 <sup>a</sup><br>± 0.08 | 4.03 <sup>a</sup><br>± 0.03 | 3.79 <sup>a</sup><br>± 0.19 | 4.00 <sup>a</sup><br>± 0.00 | 4.01 <sup>a</sup><br>± 0.01 | 4.00 <sup>a</sup><br>± 0.00 | 4.00 <sup>a</sup><br>± 0.00 | 4.00 <sup>a</sup><br>± 0.00 |
|                                       | M1 <sup>§</sup> | 4.06 <sup>a</sup><br>± 0.05 | 4.12 <sup>a</sup><br>± 0.07 | 4.60 <sup>a</sup><br>± 0.10 | 4.01 <sup>a</sup><br>± 0.01 | 5.00 <sup>b</sup><br>± 0.01 | 5.33 <sup>b</sup><br>± 0.49 | 4.13 <sup>a</sup><br>± 0.13 | 4.17 <sup>a</sup><br>± 0.17 | 4.17 <sup>a</sup><br>± 0.17 |
| BOD (ppm)                             | C <sup>§</sup>  | 5.43 <sup>a</sup><br>± 0.10 | 5.39 <sup>a</sup><br>± 0.04 | 5.61 <sup>a</sup><br>± 0.11 | 5.95 <sup>a</sup><br>± 0.36 | 5.67 <sup>a</sup><br>± 0.33 | 5.93 <sup>a</sup><br>± 0.41 | 5.03 <sup>a</sup><br>± 0.02 | 5.67 <sup>a</sup><br>± 0.33 | 5.67 <sup>a</sup> ± 0.33    |
|                                       | M1 <sup>§</sup> | 5.49 <sup>a</sup><br>± 0.12 | 5.43 <sup>a</sup><br>± 0.11 | 5.40 <sup>a</sup><br>± 0.19 | 4.16 <sup>a</sup><br>± 0.04 | 4.06 <sup>a</sup><br>± 0.03 | 5.19 <sup>a</sup><br>± 0.57 | 5.16 <sup>a</sup><br>± 0.08 | 5.02 <sup>a</sup><br>± 0.02 | 5.34 <sup>a</sup><br>± 0.34 |
| COD (ppm)                             | C <sup>§</sup>  | 157.8 <sup>a</sup> ± 1.72   | 160.3 <sup>a</sup> ± 0.38   | 147.3 <sup>a</sup> ± 6.84   | 160.1 <sup>b</sup> ± 0.18   | 140.1 <sup>a</sup> ± 0.11   | 146.7 <sup>a</sup> ± 3.33   | 140.0 <sup>a</sup> ± 0.00   | 140.0 <sup>a</sup> ± 0.00   | 140.0 <sup>a</sup> ± 0.00   |
|                                       | M1 <sup>§</sup> | 156.4 <sup>a</sup> ± 1.56   | 159.3 <sup>a</sup> ± 0.87   | 152.0 <sup>a</sup> ± 6.00   | 149.7 <sup>a</sup> ± 3.42   | 140.5 <sup>a</sup> ± 0.23   | 140.0 <sup>a</sup> ± 0.00   | 140.0 <sup>a</sup> ± 0.00   | 140.0 <sup>a</sup> ± 0.00   | 140.0 <sup>a</sup> ± 0.00   |
| Vibrio count (10 <sup>2</sup> cfu/ml) | C <sup>§</sup>  | 202.8 <sup>a</sup>          | 268.4 <sup>b</sup>          | 170.0 <sup>b</sup>          | 273.7 <sup>b</sup>          | 151.7 <sup>b</sup>          | 133.7 <sup>b</sup>          | 20.5 <sup>b</sup>           | 9.6 <sup>b</sup>            | 19.0 <sup>b</sup>           |
|                                       | M1 <sup>§</sup> | 195 <sup>a</sup>            | 115.0 <sup>a</sup>          | 89.11 <sup>a</sup>          | 69.85 <sup>a</sup>          | 71.13 <sup>a</sup>          | 72.94 <sup>a</sup>          | 10.25 <sup>a</sup>          | 5.75 <sup>a</sup>           | 15.00 <sup>a</sup>          |

§Control.

¶MasterMIN™ Aqua. All values are mean ± SD (n = 3). Values with different superscripts in a column of each parameter differ significantly (p < 0.05).



from the improved performance of animals. This affirms the fact that application of mineral mixture (M1) in low saline culture media would result in increased shrimp yield. Findings of this study revealed that

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## CONFLICTS OF INTEREST

No conflict of interest.

## AUTHOR CONTRIBUTIONS

Juliet Nesapriyam performed the calculations, analyzed the data and wrote the manuscript with input from all authors. Rahul Mathew reviewed the analyzed data. Vidya A designed the study. Rajalekshmi M aided in interpreting the results and reviewed the manuscript. Dinesh K and Geeji MT conceived the study and were in charge of overall direction and planning to conduct analysis.

## ETHICAL STATEMENT

The experimental protocol was designed in accordance to guidelines proposed and approval by the Kerala University of Fisheries and Ocean.

The study was performed in accordance with the ethical standards as laid down in the Act 5, 2011 by the Government of Kerala.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Shrimp diseases remain a major concern

Early rains falling all over the Mekong Delta when May approaches result in environmental changes and disease outbreaks. Disease monitoring results by provincial animal health care institutes and testing results from damaged shrimp ponds showed the presence of dangerous diseases, especially Microsporidian caused by *Enterocytozoon hepatopenaei* (EHP).

### Worries about disease outbreak

On May 05, Chairman of Sao Ta Foods JSC Hồ Quốc Lực told reporters of VFM about the appearance of EHP disease in some farming areas in Sóc Trăng. Deputy Head of the provincial Sub-department of Livestock production and Animal Health Đào Văn Bảy confirmed: "Observing shrimp farming areas in the province, we recognized the appearance of white spot syndrome, acute hepatopancreatic necrosis, and *Enterocytozoon hepatopenaei* with a high rate of infection, resulting in damages. Ten of the samples were positive for two diseases and four samples were positive for three diseases in three key farming areas in Vĩnh Châu town, Trần Đề district, and Cù Lao Dung district".

*>> According to reports by the Animal Health Care sub-department during the Workshop "Prevention of disease outbreak on seafood in 2022" held by the Ministry of Agriculture and Rural Development in May 07, a total of 11,000 hectares of aquaculture area have been damaged, 2.14 times as much as the same period last year. Mainly brackish water shrimp farming areas were destroyed, with 10,814 hectares being reported, 2.2 times as much as the same period last year, accounting for 2.1% of the total shrimp farms of the country. White spot syndrome, acute hepatopancreatic necrosis and EHP were found. Extensive shrimp farming ponds, which were built on natural ponds with poor rehabilitation and existing water resources, filled together with various species such as fish, mud crab, and mollusk, added with zero industrial feed, and have used traditional broodstocks in the whole year, were attacked.*

As for the EPH infection, farmer Thái Sứ Cơ in Hòa Bình district of Bạc Liêu province said that local shrimp farmers were extremely worried about EHP infection as it does not cause mortality but slow growth. Cơ said: "After EMS and white spot syndrome were well controlled, EHP emerged

and damaged shrimp. This year is no exception when large farming areas nearby have been found infected with these parasites. It is like an epidemic because if EHP is found in a pond, the auxiliary ponds will be infected just after a short time. EPH is the biggest concern, not EMS".



### PHÙNG ĐỨC TIẾN, DEPUTY MINISTER OF AGRICULTURE AND RURAL DEVELOPMENT

*In the future, the agriculture sector of provinces and cities must assign staff to effectively hold national programs and plans of animal diseases prevention approved by the Prime Minister. As in the significant seafood farming areas, the local authorities must follow up the course of the disease outbreak, catch up with updated information of the diseases and unusual death of species, take samples for testing to find out the causes, and instruct farmers with specific solutions to enhance the disease prevention. Plans of supervision for active prevention and programs of environmental monitoring must be carried out so that local farmers will be warned promptly and given specific and effective treatment methods. The quality of water resources must be improved, wastewater and charge must be handled, and sterilization must be conducted so as to prevent pathogen emergence, environmental pollution and widespread diseases.*

Not only Sóc Trăng, Bạc Liêu, but some provinces related to shrimp culture in the Mekong Delta are reported with the infection of EHP and some other dangerous shrimp diseases. According to results by the TVU Center for Product Evaluation of Trà Vinh University, ten samples sent by a customer from Duyên Hải town were positive for EHP, three samples sent by a customer from Mỹ Long Nam commune of Cầu Ngang district were also positive for EHP, and 18/22 samples sent by another customer from Duyên Hải are positive for this disease. According to shrimp farmers in Duyên Hải town, a large number of shrimps were found slow-growing and most of the samples taken from local farming areas were tested positive for EHP, pond liners or inland round ponds included.

These are concerning results because the Mekong Delta is preparing for the run-up to the main harvest. Besides this, the early crop has just been harvested and the new stocking has just been conducted for 15-50 days. In the shrimp farming area owned by the Vietnam

Clean Seafood Corporation, shrimp stocked for the first phase of the second crop of the year are now 40 days old; however, General Director Võ Văn Phục does not expect so much from this crop due to the unusual weather and disease outbreak which occurs nearly 45 days earlier than previous years. He said: "The first crop was difficult this year because of either temperature difference between day and night or the heavy off-season rain lasting over a week in March, resulting in a decrease in salinity in most of the water resources. When the rainy season starts, EHP parasites easily emerge and damage shrimps. This rule is being proven by the actual situation.

### Active prevention

EHP is an intracellular microsporidian with no specific treatment; thus antibiotics are not effective. If shrimp are found dead unusually or slow-growing after 25 days since stocking, farmers should report to local animal health care offices to find out the cause and be given proper solutions as regulated. For EHP



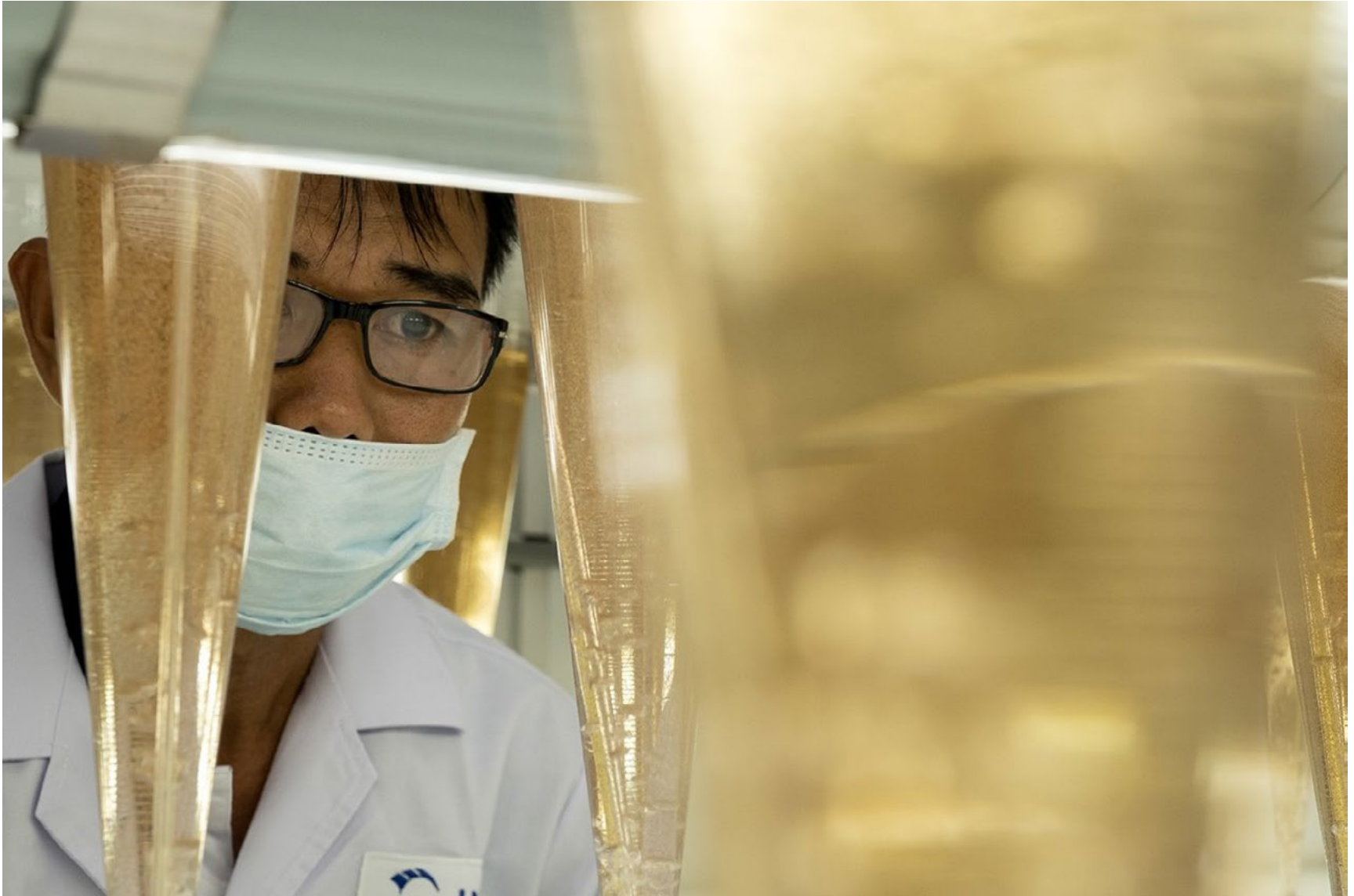
prevention, Bầy said, farmers must follow biosafety principles in managing shrimp ponds, such as: prevent strangers from entering the pond, sterilize equipment after use, filter water (when replacing or supplement water to the pond), prevent crustaceans by surrounding mesh and natural birds by proper methods, and absolutely do not move shrimp from the infected pond to the other shrimp pond so as to prevent cross-infection.

The shrimp exporting market is promising; however, according to companies, if materials are in short supply, they will step up efforts in vain. Thus, companies have been worried about the information of shrimp disease outbreak because if prompt solutions and effective treatment are not provided, material shortage is inevitable, even during the main harvest season this year. The rainy season has approached. The salinity in farming areas will continue to decrease. Unless proper solutions are not provided, a widespread disease outbreak is highly possible.

**AN XUYỀN**



# The Artemia Paradox



One of the most animated discussions in the global aquaculture community undoubtedly remains the industry's dependence on brine shrimp *Artemia* for larval rearing. Paradoxically enough, one can state that the very thing that has made large-scale aquaculture possible, if not well managed, might also be one of the greatest impediments for further growth of our industry in the future.

In a series of articles, we gathered the thoughts of some of the leading *Artemia* experts and pioneers looking at the success and adequacy of past, present and future *Artemia* feeding strategies in fish and shrimp hatcheries worldwide.

## Historic importance of *Artemia*

Historically, the major difference between some species in aquaculture and

terrestrial animal farming has always been the fact that the larvae of shrimp and most marine species have to be offered a live food, whereas chicken and cattle accept inert diets throughout their lifecycle. And because culturing of the zooplankton (the natural food of fish and shrimp larvae) was either commercially unfeasible or technically hard to realize, the efforts of early aquaculture pioneers were hampered by inadequate larval food supplies.

"The beginning of a solution to this problem came from the discovery by Seale (1933) in the USA and Rollefson (1939) in Norway, that new-born fish larvae could be fed with the 0.4 mm nauplius larva of brine shrimp *Artemia*. A small crustacean species that thrives in salty, desert like conditions where few micro algae and bacteria survive. This discovery

was particularly interesting because of the unique reproductive system of this fascinating crustacean species.

For the species to survive in its typically harsh environment, *Artemia* have developed the ability to produce 'winter eggs'. These 'eggs' are in fact cysts containing embryos that enter a period of dormancy. This is an intriguing biological event when an organism is in a deep sleep, stops metabolizing and only wakes up when conditions are favourable. This means that these encapsulated inactive embryos can be stored for a long time and only have to be incubated under the right conditions to produce free-swimming nauplii that are very well accepted as a food source for fish and shrimp larvae. All of a sudden aquaculture had theoretical access to a source of live feed that could be produced on demand."

## Development of the Artemia market

The first true commercial supply of Artemia cysts came from salt ponds in the San Francisco Bay area (California, USA) and later from the Great Salt Lake (Utah, USA). The latter one remains an example of a sustainably managed and harvested natural resource of quality cysts up to this day.

Very soon however, the increasing demand exceeded the yearly harvest of approximately 30 to 50 metric tons. From the late sixties on, there was an aggravating cyst shortage, and the hatching quality of the available cysts became less and less reliable. It was only after the FAO's Technical Conference on Aquaculture in 1976 in Kyoto that the situation started to change for the better.

Mandated by the FAO to do so and based on his earlier work at the University of Ghent, at that time Belgian biologist and researcher Patrick Sorgeloos led a number of initiatives from 1978 on to turn the limited global Artemia supply into a future proof resource for professional aquaculture.

This work later resulted in the foundation of Artemia Reference Center and of INVE Aquaculture. The work done by Patrick Sorgeloos and his team focused on finding solutions for the major impediments for the further development of Artemia use in aquaculture: The need to explore new natural sources of Artemia in Europe, Asia, North and South America, and Australia and the introduction of brine shrimp in other suitable habitats such as North-East Brazil, Vietnam and China.

Improvement of the cyst quality by developing new harvesting techniques such as harvesting at the water surface instead of at the lake shores where dirt particles build up between the cysts and the repeated hydration-dehydration cycles affect the hatching quality and energetic content of the embryos. Improvement in terms of yield reliability by developing standard Artemia hatching protocols that establish the optimal conditions for the nauplii to hatch successfully and simultaneously. Classification and fingerprinting of the differences in size, hatching performance, growth rate, nutritional value etc. of Artemia cysts coming from different strains, locations and even harvests.

## From 'raw' Artemia to Artemia products

For a long time Artemia cysts were simply harvested from the shoreline and distributed as a 'raw' product. But due to seasonal and regional variations, differences between sub-species, contamination of the product with sand and other external substances, the



Philippe Léger - Former CEO of INVE Aquaculture

efficient and safe use of Artemia as a live feed required a more structured and responsible approach.

"Decades of intensive research and development have led to the sophisticated live feed resource aquaculture knows today: a carefully processed product, namely the dry cysts that produce live nauplii within 24 hours after incubation. Thanks to superior processing and fingerprinting techniques, cysts can be packed and labelled according to their specific characteristics such as size, hatching capacity and nutritional value, regardless of their strain or origin.

Advanced and patented technologies make it possible to break the embryos' dormancy on demand for optimal hatching efficiency, to easily separate the empty cyst shells from the nauplii after hatching and to secure the Artemia's biosecurity and energetic content with innovative processes and enrichment (Selco) products. This means that in an ideal situation every hatchery can procure the exact Artemia that is most suited for its specific application needs, without having to dread any kind of unpredictable outcome." – Alessandro Moretti

## Current situation and bottlenecks

Today we are at a point where the world's most interesting Artemia resources have been identified and are exploited to their full capacity while maintaining a sustainable harvesting rate. All together these sources provide a global supply of more than 3,000 metric tons per year. This is a major industry bottleneck on its own.

Because, unlike global aquaculture which is expected to keep on growing exponentially, the Artemia supply will remain more or less stable at its current capacity.

## Patented technologies in Aquaculture: blessing or curse?

The considerable ongoing investment in the research and development (R&D) of new technologies continues to open up exciting possibilities to improve the management and productivity of hatcheries and farms worldwide. However, to protect this investment in R&D, often the companies' investing in these new innovations will patent the technology. How should we look at this as an industry? The discussion arose once more recently, when Benchmark Holdings (representing its subsidiary INVE Aquaculture) won a case before the IP&IT court in Bangkok to protect its patents relating to its Artemia hatching technologies.

The company successfully prosecuted infringement of its patented technology in Thailand. In this case, the patents specifically applied to technology that allows INVE to break the naturally long and unpredictable dormancy of certain Artemia cysts. The patented treatment elevates these cysts' normal hatching percentage of approximately 20-40% to 80+% and therefore has brought enormous benefits to INVE Aquaculture's customers and the market.



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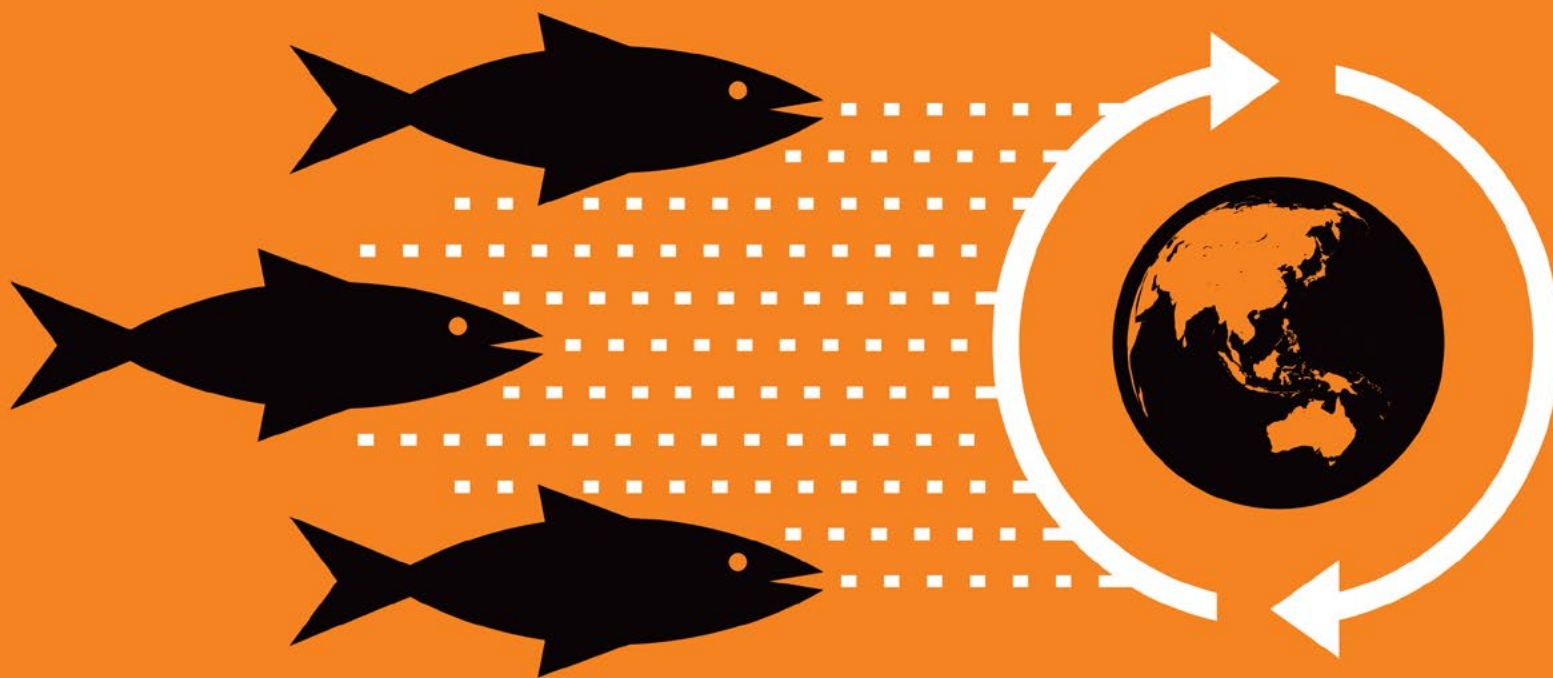


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Effective, efficient mariculture operations also have the benefit of requiring only a small amount of space; according to the study, typically 3 percent or less of a country's exclusive economic zone is required to meet consumer demand © Australis Aquaculture

# Study backs huge untapped potential of marine finfish farming in an era of climate change

Choosing the right species and locations to farm will allow farming of marine finfish to thrive, even in an period of changing ocean temperatures, oxygen levels and salinities.

So argues an international collaboration of researchers, in a new paper, published in the journal *Nature*, who look at the potential of a warming ocean to satisfy the growing global appetite.

“Climate change will challenge the ability for the ocean to meet the seafood demands of a growing population,” said Christopher Free, a researcher at the UC Santa Barbara and lead author of the paper, in a press release. “However the ocean could produce more food than today through swift and ambitious actions.” Currently providing only 17 percent of the world's protein supply, most of it from capture fisheries, the ocean holds great potential to help satisfy the global demand for meat, which is expected

to keep growing – especially as developing countries grow richer. But it's also subject to global greenhouse gas emissions, which results in effects such as warming and acidification – phenomena that are expected to decrease the ocean's ability to meet the seafood demands of a growing population.

“Warming waters are changing where fish can live, what prey they can eat, and how well they can survive,” said Free. “Both fishers and fisheries managers have to adapt to these changes.” Fisheries can maintain or increase their yields by adapting their practices to shifts in the productivity and location of their fish stocks, the researchers said. However, these reforms alone, though necessary, will be insufficient to

fulfill future demand. Finfish and shellfish farms will be essential to fill that gap. “Expansion of sustainable ocean aquaculture could build on fisheries reforms to increase the availability of healthy and sustainable seafood to our growing population,” said Halley Froehlich, an assistant professor at UC Santa Barbara and co-author of the paper. According to the study, the expansion of mariculture is projected to be limited by consumer demand or availability of feed ingredients derived from wild fisheries, rather than by climate change. With the appropriate selection of species and location, for example, the researchers found that “the availability of area for profitable finfish mariculture to be insensitive to changing temperature, oxygenation and salinity.”



Fish farming near Amarnythos, Euboea, Greece

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Additionally, because food production by its nature generates impacts to the environment, the sustainable expansion of mariculture must be conducted carefully. “It will require improved governance to ensure best practices that minimize impacts on ocean ecosystems and encourage equitable access to this growing industry,” said Willow Battista, a senior manager at the Environmental Defense Fund and also a co-author on the paper.

What this sweet spot of governance looks like will vary by location. In highly regulated

geographies such as the United States, mariculture regulations may need to be more well defined to allow sustainable mariculture to grow. In weakly regulated regions, such as China or Thailand, standards will have to be maintained to prevent inefficiencies and ecosystem degradation. Regions where little or no historical mariculture production exists, as in many of the African nations expected to be hardest hit by climate change, will have to invest in training and infrastructure.

### More efficient aquaculture

Effective, efficient mariculture operations also have the benefit of requiring only a small amount of space; according to the study, typically 3 percent or less of a country’s exclusive economic zone is required to meet consumer demand. “The small space requirement for mariculture leaves ample room for careful planning to minimize impacts on other ocean industries,” said Steve Gaines, dean of the Bren School and a co-author on the paper. With careful coordination, mariculture operations can be set up so as not to compete with fisheries, coastal tourism and shipping, among other ocean industries.

In fact, its efficiency is one of mariculture’s key advantages: it has a lower greenhouse gas

footprint and lower demands for water and land than many land-based sources of meat. And with improvements to finfish feed, breeding and husbandry, it is possible to further increase yield to meet consumer demand. According to the researchers, gearing food production toward the ocean could not only satisfy the growing global appetite for meat, it could also free up land required for terrestrial agriculture. “Shifts in consumer preferences away from terrestrial meat could reduce the environmental impacts of global food systems,” Froehlich said. With an increase in production, prices are expected to decrease, allowing access to local, sustainable and nutritious food.

Producing food from the ocean for future populations will no doubt take a global concerted effort. But nowhere is it more urgent than in tropical developing countries, places where climate driven productivity loss, coupled with growing populations, put a great strain on food security. “Tropical developing countries will endure the greatest losses in fisheries catches and may therefore be priorities for investments in ocean aquaculture expansion,” said Reniel Cabral, a senior lecturer at James Cook University and co-author on the study.

# The Vietfish Interview

**DR. LOC TRAN, FOUNDER & DIRECTOR, SHRIMPVET**

## 1. What encouraged you to enter aquaculture?

I grew up in the early 80s, when Vietnam was still quite isolated and suffering economically due to the sanctions. There was only nature and fish and paddy fields, and I was in love with aquatic species – fish and shrimp were like my pets. It also helped that my dad was a professor of aquaculture at the university and although most of the fish available was wild-caught, they had a hatchery for carp and tilapia and I was aware that aquaculture was an important part of the economy, supporting millions of households, although only in the last 20-25 years did professional, rather than subsistence, aquaculture, emerge.

## 2. What secret do you have to build Shrimp Vet as today?

In the past 4-5 years there have been so many innovations. ShrimpVet's rise has coincided with a dramatic increase in shrimp production in Vietnam and the country's annual shrimp production has risen by 10-15 per cent a year over the last three years.

I have my enthusiasm for genetics, I have collaborated with broodstock companies to improve the genetic lines of his post-larvae (PL). And I'd like to ramp this up. I'd love to collaborate more closely to improve traits such as growth rates and disease-resistance and would, ultimately, like to be vertically integrated so we can control everything from genetics, to disease control, to farming protocol. The company has the capacity to produce 1.5 billion SPF PL a year.

Despite its name, ShrimpVet works with a variety of species, including tilapia, pangasius and barramundi, with a focus on nutrition and health. And, despite its roots in Vietnam, I am looking to work more widely in other countries too.

## 3. What are most important means to negate the need to use antibiotics in shrimp production?

The key place to start is the need to apply biosecurity in your production unit, and that can be done by better water treatment,



biosecurity measures, diagnostics and being sure that the facility is pathogen free.

Bearing in mind that we have to deal with the ubiquity of vibrios and other types of bacteria, the second step is to keep the environment clean – through better pond management and also water treatment and removing sludge and effluent during culture. It's also crucial to use probiotics to take care of the waste – organic waste and also other kinds of effluents like ammonia, nitrite and hydrogen sulphide. By keeping the environment clean you will have clean stock and clean water, and the animal will be very healthy.

We also need to keep the aquatic animal's gut microbiota healthy by applying active probiotics for microbiome moderation. By doing that, vibrios cannot produce enough toxins to cause damage to the animals.

In the feed itself we can apply feed additives, prophylactics and quorum-quenching products to minimise the impact of bacterial infection. Particular ingredients include acidifiers, monoglycerides and phytogenics, which provide a good substrate

to replace the use of antibiotics because they can suppress the growth of harmful bacteria and promote a healthy microbiome in the animal gut.

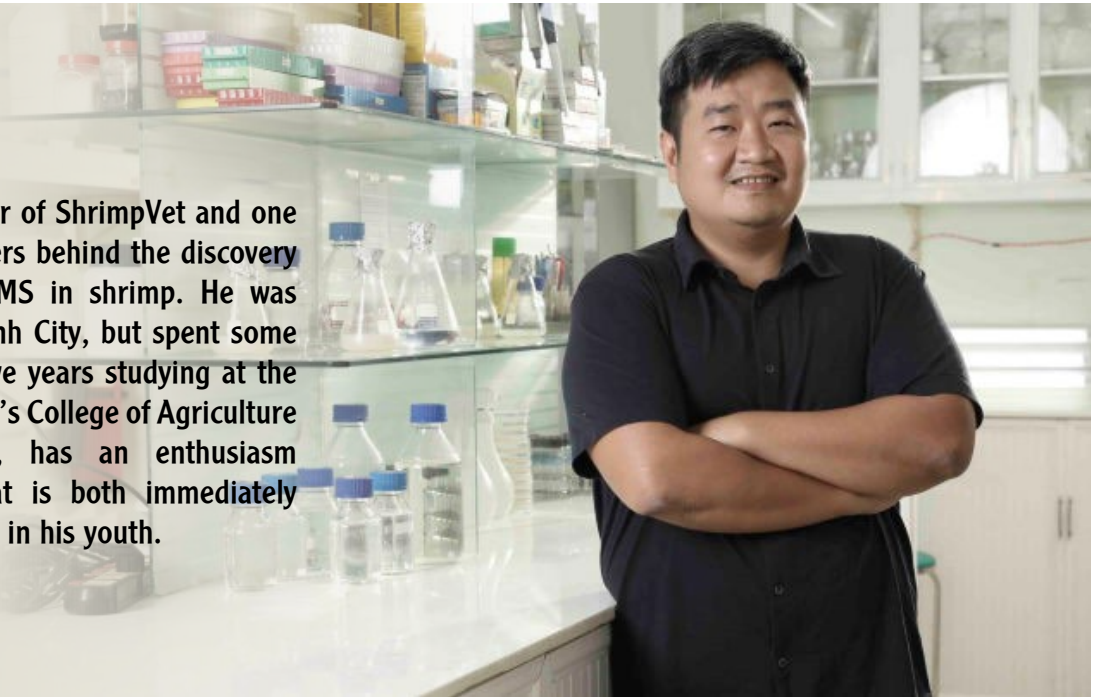
All of these methods are only effective, however, if farmers are well educated and well informed about new developments in farming technology so that they're able to see the broader picture. They also must have a very clear farming protocol from day one.

## 4. How has applying these techniques affected your own production?

The ShrimpVet lab was founded about nine years ago and our main activities were initially in R&D and diagnostics. However, we soon began to realise that in order to better assist farmers we had to apply those concepts into shrimp production. This is why we founded a hatchery – it gave us the chance to start applying these concepts of biosecurity, probiotic maturation in the water, probiotics in feed and better management of the environment. This allowed us, eventually, to produce very high-quality shrimp post-larvae and we can now apply the same concept to our grow-out farm too – we now have a pretty decent sized farm, all our ponds now plastic-lined and we apply probiotic activation



Dr Loc Tran, founder of ShrimpVet and one of the key researchers behind the discovery of the causes of EMS in shrimp. He was raised in Ho Chi Minh City, but spent some of his most formative years studying at the University of Arizona's College of Agriculture and Life Sciences, has an enthusiasm for aquaculture that is both immediately apparent and rooted in his youth.



from the nursery phase, as well as all the disease-control concept lines mentioned earlier.

### 5. How has this worked out for you?

Everything has become very consistent – the survival rate at our hatchery is always about 50 percent, we don't have any issues with luminescent disease or EMS [early mortality syndrome] or other bacterial diseases in the hatchery. At the grow-out farm level we don't encounter either EMS or white faeces when we apply a clear farming protocol with proactive disease-control measures.



### 6. Do you have any projects or plans that we should look out for over the coming year from you and your company?

In early 2021, we have teamed up with Signify to investigate whether the addition of lighting can improve the stability of shrimp farming systems and produce more consistent shrimp yields. We trialled Philips AquaAdvance 260W Shrimp lights, which had been tailored towards shrimp using a patented blue and green spectrum. Adding lighting to shrimp production makes the shrimp grow faster and increase the survival rate by 35 percent. I saw this as a huge potential to increase shrimp production. Applying technologies such as lighting would increase shrimp's growth and resistance to weather and seasonal changes. The

use of artificial lighting prevents abrupt light changes that can trigger stress reactions in shrimp. It also allows farmers to control environmental conditions and influence shrimp's behaviour, such as feeding activity, and physiological processes including metabolic rate, growth and maturation. On 30<sup>th</sup> March this year, we have launched the workshop in Ho Chi Minh city to announce this new project with Signify. The Signify/ShrimpVet research collaboration continues and grows in this year.

## NEW INTERNATIONAL SHRIMP RESEARCH

# Development of new real-time PCR methods for detection of Decapod iridescent virus 1 in shrimp

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Australian company Genics are 100% committed to developing & delivering novel tools and commercial access to the global Shrimp industry promoting improved shrimp health management & biosecurity. As part of this commitment, their R&D Team has produced NEW research on Decapod Iridescent Virus (DIV1) real-time PCR assays published as *free open access* for all in the global shrimp industry. This scientific research article is a timely reminder for continuous shrimp pathogen surveillance and disease mitigation via proven next-generation technologies such as Genics' Shrimp **MultiPath Xtra**.

Decapod Iridescent virus (DIV1) infections emerged in mainland China around 2014 and have devastated shrimp aquaculture operations in Chinese coastal provinces. In 2020, DIV1 has spread to Taiwan with devastating results to shrimp and crayfish farms, in addition to being found in wild caught *Penaeus monodon* from the Indian Ocean. This trend is a major cause for concern and an urgent reminder to expand the tools needed to monitor the spread of DIV1 globally.

In this paper, we describe a set of four different real-time polymerase chain reaction (PCR) assays positioned across the genome of DIV1 to detect the virus in shrimp tissues. All four assays show a wide dynamic range and high analytical sensitivity and specificity. In addition, the newly developed assays show excellent diagnostic sensitivity and specificity in clinical *Litopenaeus vannamei* samples of North Asian origin. The new molecular toolset will enhance global capabilities to monitor the spread of DIV1 and ultimately be used as an early warning system for farmers and authorities to engage in appropriate risk mitigation strategies.

## 1. Introduction

Since it was first mentioned in 1993 by two independent groups (Lightner & Redman, 1993; Montanie, Bonami, & Comps, 1993), suspected Iridovirus infections in members of the order Decapoda were only reported by Tanget al. (2007) within a timeframe of 20 years. Yet, the identification, isolation, and genomic characterization of two new pathogenic iridoviruses in redclaw crayfish *Cherax quadricarinatus* (*Cherax quadricarinatus* iridovirus [CQIV]) (Li, Xu, & Yang, 2017; Xu, Wang, Li, & Yang, 2016) and in *Litopenaeus vannamei* (Shrimp hemocyte iridescent virus [SHIV]) in December 2014 (Qiu et al., 2017; Qiu, Chen, Wang, et al., 2018) seemed to mark the re-emergence of a concerning outbreak trend of iridovirus infections in South-East Asia. Phylogenetic assessment of genomic and deduced protein sequence data led to the reclassification of the newly identified iridoviruses CQIV and SHIV to be part of the new genus

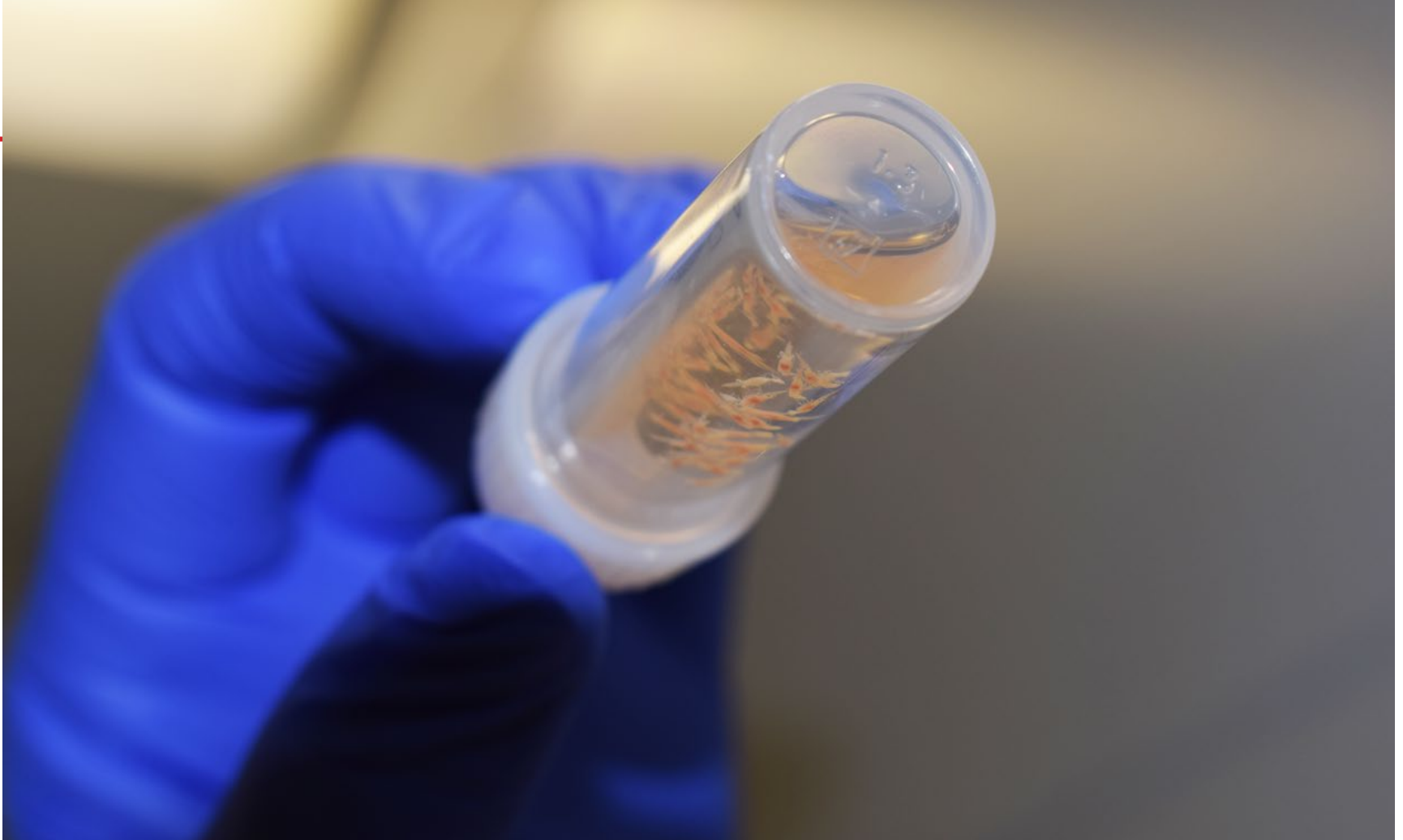
## Abstract

Novel Decapod Iridescent virus (DIV1) infections emerged in mainland China around 2014 and have devastated shrimp aquaculture operations in Chinese coastal provinces. In 2020, DIV1 has spread to Taiwan with devastating results to shrimp and crayfish farms, in addition to being found in wild caught *Penaeus monodon* from the Indian Ocean. This trend is a major cause for concern and an urgent reminder to expand the tools needed to monitor the spread of DIV1 globally. Here, we describe a set of four different real-time polymerase chain reaction (PCR) assays positioned across the genome of DIV1 to detect the virus in shrimp tissues. All four assays show a wide dynamic range and high analytical sensitivity and specificity. In addition, the newly developed assays show excellent diagnostic sensitivity and specificity in clinical *Litopenaeus vannamei* samples of North Asian origin. The new molecular toolset will enhance global capabilities to monitor the spread of DIV1 and ultimately be used as an early warning system for farmers and authorities to engage in appropriate risk mitigation strategies.

## Keywords

*Decapod iridescent virus 1, Iridovirus, real-time PCR, Shrimp MultiPath Xtra*

*Decapodiridovirus* in the family Iridoviridae (Chinchar et al., 2018). In fact, both CQIV and SHIV were assessed to be different strains of the same viral species *Decapod iridescent virus 1* (DIV1). Both DIV1 viruses contain a linear double-stranded DNA genome with a size of 165,695 bp (CQIV) and 165,809 bp (SHIV) (Liet al., 2017; Qiu, Chen, Wang, et al., 2018). DIV1 infects hematopoietic tissue, gills, and hepatopancreas of its host and leads to symptoms like empty stomach, a degree of discoloration on the surface of the hepatopancreas and softening of the shell leading to an overall high mortality of infected individuals (Qiu et al., 2017; Xu et al., 2016).



While deadly DIV1 outbreaks were mainly confined to coastal provinces of China (Kearns, 2020), it has since spread to shrimp and crayfish farms in Taiwan (The Fish Site, 2020). Interestingly, DIV1 has recently been detected in wild caught *Penaeus monodon* broodstock specimens in the Indian Ocean without any clinical signs of disease (Srisala et al., 2021). This in turn is lending to the concern that *P. monodon* might potentially be a host for DIV1 with the inherent risk to spread this emerging pathogen throughout the Asia-Pacific region.

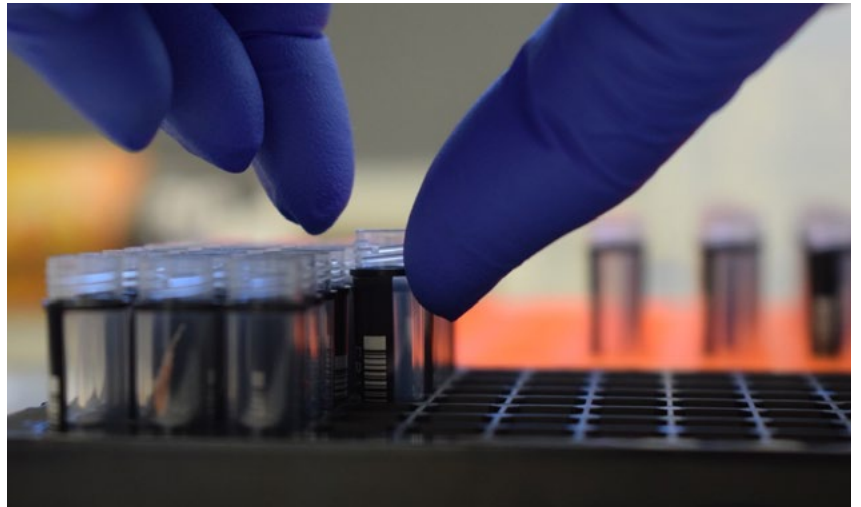
Several PCR-based molecular methods targeting a limited number of viral hypothetical genes have been described (Table 1). Qiu et al. (2017) and Srisala et al. (2021) have opted to use nested PCR approaches targeting the viral ATPase gene and the major capsid protein (MCP) to detect the virus. Both currently available TaqMan real-time PCR approaches are targeting the viral ATPase gene (Gong et al., 2021; Qiu, Chen, Wan, et al., 2018). Two loop-mediated isothermal amplification methods (LAMs) described by Chen et al. (2019) and Gong et al. (2021) are detecting the RNA polymerase II and ATPase, respectively, while a recently published recombinase polymerase amplification method focuses on MCP (Chen et al., 2020).

This study aims to expand the molecular tools portfolio for DIV1 detection with three novel SYBR real-time PCR assays directed at three new target genes spreading across the assembled genome from shrimp hemocyte iridescent virus isolate 20141215 (MF599468; Table 2). Based on the assembly, the putative target genes are ORF 51R (putative papainase gene), ORF 124R (putative cell surface gene), and ORF 114R (putative D5 family NTPase ATPase gene), which are also used in other studies referenced above. These new real-time PCR assays provide a broader gene target assay range for biosecurity agencies, regulators, and the global shrimp industry to better monitor and mitigate the risks posed by this emerging pathogen, DIV1.

## 2. Materials and methods

### 2.1 Sample collection and nucleic acid extraction

*L. vannamei* pleopod samples were collected from live individual shrimp taken from an earthen pond suffering mortalities in Northern Asia. Shrimp samples were submerged in 70% Laboratory Grade Ethanol for preservation



and sub-sequent total nucleic acid (TNA = RNA and DNA) extraction. TNA was extracted using a MagMAX™ Core nucleic acid purification kit with the KingFisher FLEX robot (Thermo Fisher Scientific, CA) as described by Moser, Franz, Firestone, and Sellars (2022). Extracted sample TNA was eluted in 50 µl PCR grade water and directly used for the different analyses.

### 2.2 PCR-based assay design and run parameters

A total of 10 real-time PCR assays targeting various open reading frames (ORF) from SHIV isolate 20141215 (Accession # MF599468) were selected for prescreening based on the following criteria:

- Putative function (ranging from potential pathogenicity to exotic functions)
- Distribution across the genome map of SHIV isolate 20,141,215 (Accession MF599468)
- BLAST analysis for each potential target ORF

All PCR assays developed in this study were designed using the PRIMER 3 software (Untergasser et al., 2012) as implemented in the NCBI interface (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>)

index.cgi?LINK\_LOC= BlastHome). The final selection of four SYBR green based real-time PCR assays presented here target ATPase gene (DIV ATPase), patatin (DIV PAT), papainase (DIV PAP), and cell surface G (DIV CSG) and were chosen based on amplification efficiency, titration curve fit, linear dynamic range and melt curve analysis. These assays were further developed as diagnostic assays for monitoring DIV1 infections. Assays and corresponding primer sequences used in this study are listed in Table 2.

Real-time PCR assays were setup in 384-well PCR plates using the PowerUp SYBR Green Master Mix and run on the QuantStudio 12K Flex Real-Time PCR system (Thermo Fisher Scientific, CA) described elsewhere (Moser et al., 2022).

### 2.3 Direct amplicon sequencing and sequence analysis

Real-time PCR amplicons were sequenced directly to confirm sequence authenticity. PCR amplicons in original amplification reaction mix/volume and corresponding forward and reverse primers (3.2 µM) were submitted to the Australian Genome Research Facility (AGRF, Brisbane Australia) for direct Sanger sequencing using Big Dye Terminator chemistry 3.1 and ABI Capillary Sequencer 3730xl (Thermo Fisher Scientific, CA).

The quality of sequence traces were checked and curated using the Sequencher Software (GeneCodes, MI) and verified sequences confirmed using the BLAST tool interface of NCBI (<https://www.ncbi.nlm.nih.gov/home/about/>; National Centre for Biotechnology Information, MD).

### 2.4 Assay verification and validation

Real-time PCR assays and the commercially run Shrimp MultiPath™ service (Genics Pty Ltd, Brisbane Australia) were assessed against a list of performance criteria such as serial dilution and amplification efficiency assessment (qPCR), analytical parameters, and diagnostic parameters in line with the validation pathway recommended by the OIE (OIE, 2021).

#### 2.4.1 qPCR standard curve and amplification efficiency

Synthetic double-stranded DNA templates (GBlocks; Integrated DNA Technologies, IA) for each real-time PCR assay were sourced from Integrated DNA Technologies and diluted in a 10-fold dilution series spanning 10,000 copies per reaction down to 1 copy per reaction. Four replicates per dilution step were run and amplification efficiencies calculated using Equation (1).

$$\text{Efficiency} = 10^{\frac{-1}{\text{slope}}} - 1$$

Slope is the log-linear phase of the standard titration curve.

#### 2.4.2 Analytical sensitivity and specificity

ASE, or the limit of detection (LOD), was determined via a dilution to extinction experiment and subsequent probit regression analysis. Synthetic templates for each corresponding qPCR assay were diluted in nonsymmetrical steps to reach extinction of the template in the lowest dilution steps. The following dilution steps were assessed (in copies per reaction): 100/50/25/12.5/10/6.25/5/2.5/1/0.5/0.1/0.05/0.005.

LOD was calculated via a probit regression analysis using MedCalc® Statistical Software version 20.019 (MedCalc Software Ltd, Ostend Belgium) and Equation (2) below.

$$\text{probit}(p) = a + b \log_{10} X$$

probit (p) = inverse standard normal cumulative distribution function  $\Phi^{-1}$

X = dose variable

a = regression coefficient Constant

b = slope of regression equation.

ASp was continuously assessed on synthetic template in clinical sample matrix background. Clinical samples used in this study additionally had a background of different pathogens, including Pir A toxin gene & Pir B toxin gene (AHPND/EMS; acute hepatopancreatic necrosis disease/Early mortality syndrome) and *Enterocytozoon hepatopenaei* (EHP) as confirmed by Shrimp MultiPath™ (Genics Pty Ltd, Brisbane, Australia). In addition, DIV1 qPCR assays were run against selected *L. vannamei* and *P. monodon* samples from two geographically diverse locations (Kenya and Vietnam; data not shown) with in-house confirmed hepatopancreatic parvovirus (HPV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), monodon baculovirus (MBV), and Laem Singh virus (LSNV). Assays were also tested for cross-reactivity on polychaete worms from the genus *Perinereis* sourced from South-East Queensland, Australia.

#### 2.4.3 Diagnostic specificity and sensitivity

To estimate diagnostic parameters diagnostic specificity (DSp) and diagnostic sensitivity (DSe) for all four assays, a generic golden reference standard method was employed via the interface MICE (<http://mice.tropmedres.ac/home.aspx>) on a *L. vannamei* shrimp population of 91 specimens. The upper Ct range of each assay was chosen as LOD and cut-off for binary conversion of qPCR values. Each assay was chosen to serve as assumed gold reference standard and DSe and DSp calculated for subsequent comparison.

## 3. Results

The initial step of the assay verification and validation pathway was to measure the performance of each assay in a standard curve titration analysis. Key metrics are curve fit (R-square values) showing quality of technical replicates and amplification dynamic as well as slope of the regression line to calculate PCR efficiencies. All four novel assays for the detection of DIV1 (DIV-CSG, -PAT, -PAP, and -ATPase) show a tight curve fit with R-Square values greater than 0.9. Further validated with respect to their analytical performance. For analytical specificity (ASp) samples positive for DIV1 were confirmed positive (100% inclusivity) and direct amplicon sequencing and database interrogation employed to validate the confirmed results (Table 4). Moreover, *L. vannamei* and *P. monodon* samples negative for DIV1 but positive for other shrimp pathogens such as HPV, IHHNV, MBV, LSNV, *Vibrio parahaemolyticus* (PirA/PirB) and EHP showed no false positive DIV1 assay results (exclusivity). No cross-reactivity of all new qPCR assays has also been confirmed with polychaete worm samples from the genus *Perinereis* (data not shown).

Analytical sensitivity or LOD was determined to be 2.2 copies/reaction (Ct = 32.0; DIV PAT), 2.9 copies/reaction (Ct = 32.1; DIV CSG), 3.1 copies/reaction (Ct = 31.3; DIV PAP), and 8.6 copies/reaction (Ct = 32.2; DIV ATPase) (Table 5).

Diagnostic metrics sensitivity (DSe) and DSp were based on the traditional golden reference assay approach with each assay evaluated as golden reference point to determine the differential DSe and DSp values for each of the novel DIV1 assays using a single population of 91 *L. vannamei* shrimp samples known to be infected with DIV1. The binary qPCR profile for all samples (Table 6) was established and formed the basis for the DSe/DSp assessment. With DIV ATPase set as golden reference assay 100% DSe/100% DSp, the results for the other assay were obtained as 97%/93.1% (DIV CSG), 93.9%/93.1% (DIV PAP), and 100%/93.1% (DIV PAT), respectively. Setting DIV CSG as reference 100% DSe/100% DSp, all comparative assays were 88.9%/98.2% (DIV ATPase), 91.7%/96.4% (DIV PAP), and 97.2%/96.4% (DIV PAT). DIV PAP as reference yielded DSe/DSp results 88.6%/96.4% for DIV ATPase, 94.3%/94.6% for DIV CSG, and 97.1%/94.6% for DIV PAT. Finally, DIV PAT as golden reference assay resulted in a DSe/DSp of 89.2%/100% for DIV ATPase, 94.6%/98.1%, for DIV CSG, and 91.9%/98.1% for DIV PAT.

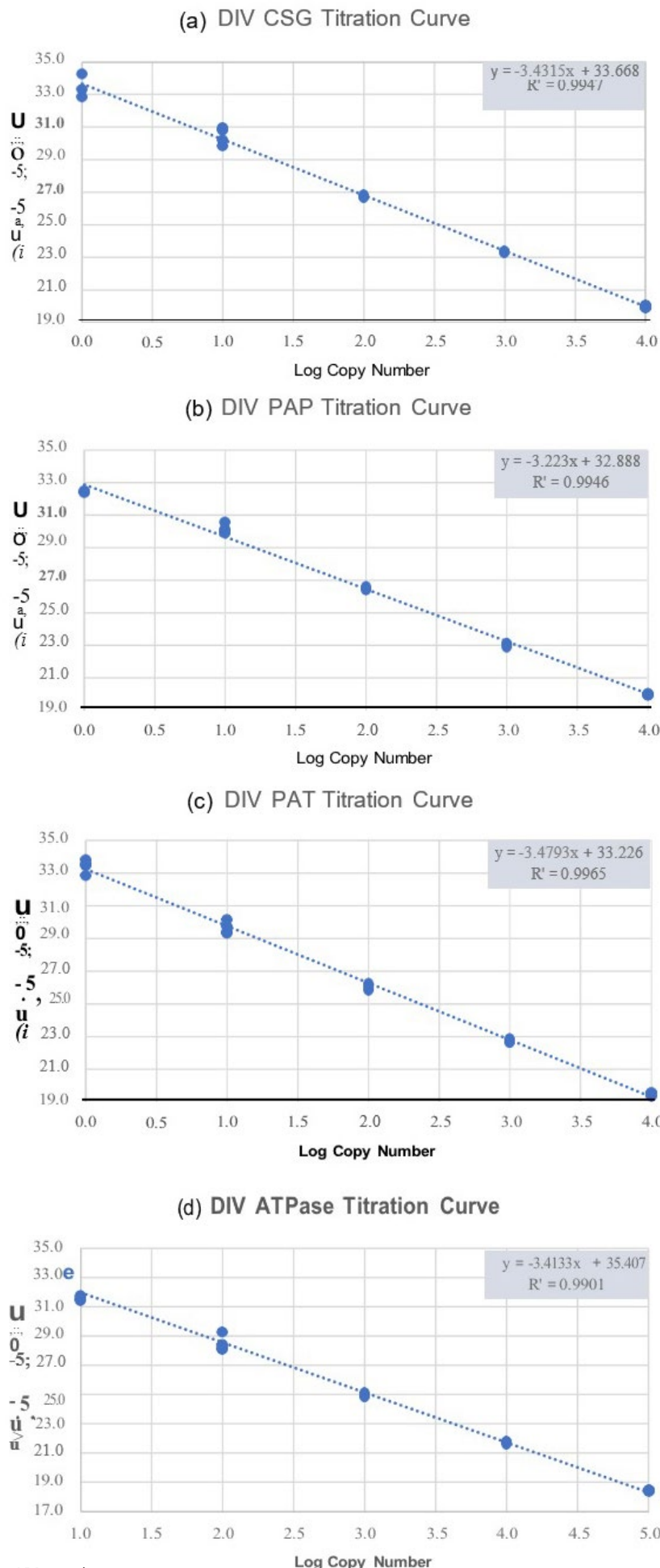


FIGURE 1. Legend on next page

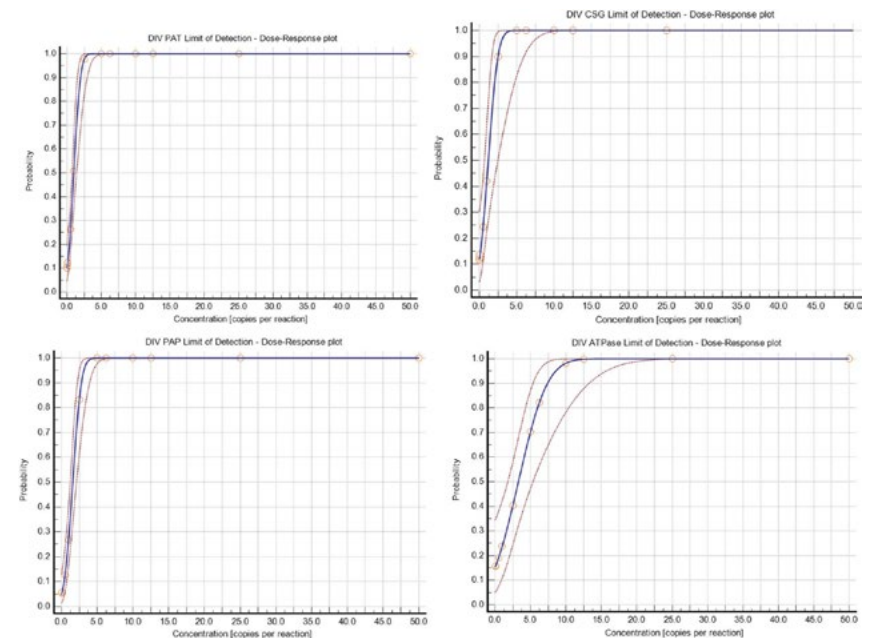


FIGURE 2. Probit regression analysis curve fit with 95% confidence interval (dotted line) shown to determine the limit of detection (LOD) for Decapod Iridescent virus patatin (DIV PAT), papainase (PAP), cell surface G (CSG), and ATPase. Sixteen replicates were tested at each concentration point and dose (cut-off copy number per reaction) determined at probability of 0.95

## 4. Discussion

The sudden occurrence of mass mortality among farmed Penaeid shrimp caused by DIV1 infections in China in 2014 (Li et al., 2017; Qiu et al., 2017; Xu et al., 2016) sparked an urgent need to detect and monitor the infectious agent DIV1 to protect the global shrimp farming industry from this emerging biosecurity risk. This included known and potential risks such as uncooked commodity shrimp and fresh frozen polychaetes used in shrimp maturation diets.

A handful of DIV1 target assays against a limited amount of target loci have been described (Table 1) leaving an acute need for more tools to detect and monitor the virus spread worldwide. Qiu et al. (2017) and Srisala et al. (2021) have opted to use nested PCR approaches targeting the viral ATPase gene and the MCP to detect the virus. Both currently available TaqMan real-time PCR approaches are targeting the viral ATPase gene (Gong et al., 2021; Qiu, Chen, Wan, et al., 2018). Two loop-mediated isothermal amplification methods (LAMP) described by Chen et al. (2019) and Gong et al. (2021) are detecting the RNA polymerase II and ATPase, respectively, while a recently published recombinase polymerase amplification method focuses on MCP (Chen et al., 2020).

The assays DIV PAT, DIV PAP, and DIV CSG, developed in this study are aimed at expanding the assay and target portfolio for DIV1 detection and monitoring (Table 2). A separate DIV ATPase qPCR assay was also developed in this study as a strong primer dimer peak was observed when running the TaqMan capture primers published by Qiu, Chen, Wan, et al. (2018) in SYBR and melt-curve analysis mode (data not shown).

All four SYBR qPCR assays showed tight titration curve characteristics across a wide dynamic range spanning 1–10,000 copies of template per reaction (range expanded to  $10^6$  copies per reaction—data not shown) with close to perfect amplification efficiencies ranging from 93.8 to 104.3% (Table 3; Figure 1). The LOD for DIV PAT at 2.2, DIV CSG at 2.9, DIV PAP at 3.1, and DIV ATPase at 8.6 copies per reaction (Tables 2 and 4, Figure 2) indicates that all assays are highly sensitive compared to reported TaqMan and LAMP assays available (Table 1). The LOD of the TaqMan assays was reported at slightly higher levels compared to the four new assays with 4 copies per reaction (Qiu, Chen, Wan, et al., 2018) and 19 copies per  $\mu$ l (Gong et al., 2021). In addition, the LOD for

| Parameters          | Test A assumed perfect gold standard (%) <sup>a</sup> | Test B was assumed as a perfect gold standard (%) <sup>a</sup> | Test C was assumed as a perfect gold standard (%) <sup>a</sup> | Test D was assumed as a perfect gold standard (%) <sup>a</sup> |
|---------------------|-------------------------------------------------------|----------------------------------------------------------------|----------------------------------------------------------------|----------------------------------------------------------------|
| Prevalence          | 36.3 (26.6–47.1)                                      | 39.6 (29.6–50.4)                                               | 38.5 (28.6–49.3)                                               | 40.7 (30.6–51.5)                                               |
| DIV ATPase (Test A) |                                                       |                                                                |                                                                |                                                                |
| Sensitivity         | 100                                                   | 88.9 (73.0–96.4)                                               | 88.6 (72.3–96.3)                                               | 89.2 (73.6–96.5)                                               |
| Specificity         | 100                                                   | 98.2 (89.0–99.9)                                               | 96.4 (86.6–99.4)                                               | 100 (91.7–100)                                                 |
| PPV                 | 100                                                   | 97.0 (82.5–99.8)                                               | 93.9 (78.4–98.9)                                               | 100 (87.0–100)                                                 |
| NPV                 | 100                                                   | 93.1 (82.5–97.8)                                               | 93.1 (82.5–97.8)                                               | 93.1 (82.5–97.8)                                               |
| DIV CSG (Test B)    |                                                       |                                                                |                                                                |                                                                |
| Sensitivity         | 97.0 (82.5–99.8)                                      | 100                                                            | 94.3 (79.5–99.0)                                               | 94.6 (80.5–99.1)                                               |
| Specificity         | 93.1 (82.5–97.8)                                      | 100                                                            | 94.6 (84.2–98.6)                                               | 98.1 (88.8–99.9)                                               |
| PPV                 | 88.9 (73.0–96.4)                                      | 100                                                            | 91.7 (76.4–97.8)                                               | 97.2 (83.8–99.9)                                               |
| NPV                 | 98.2 (89.0–99.9)                                      | 100                                                            | 96.4 (86.4–99.4)                                               | 96.4 (86.4–99.4)                                               |
| DIV PAP (Test C)    |                                                       |                                                                |                                                                |                                                                |
| Sensitivity         | 93.9 (78.4–98.9)                                      | 91.7 (76.4–97.8)                                               | 100                                                            | 91.9 (77.0–97.9)                                               |
| Specificity         | 93.1 (82.5–97.8)                                      | 96.4 (86.4–99.4)                                               | 100                                                            | 98.1 (88.8–99.9)                                               |
| PPV                 | 88.6 (72.3–96.3)                                      | 94.3 (79.5–99.0)                                               | 100                                                            | 97.1 (83.4–99.9)                                               |
| NPV                 | 96.4 (86.6–99.4)                                      | 94.6 (84.2–98.6)                                               | 100                                                            | 94.6 (84.2–98.6)                                               |
| DIV PAT (Test D)    |                                                       |                                                                |                                                                |                                                                |
| Sensitivity         | 100 (87.0–100)                                        | 97.2 (83.8–99.9)                                               | 97.1 (83.4–99.9)                                               | 100                                                            |
| Specificity         | 93.1 (82.5–97.8)                                      | 96.4 (86.4–99.4)                                               | 94.6 (84.2–98.6)                                               | 100                                                            |
| PPV                 | 89.2 (73.6–96.5)                                      | 94.6 (80.5–99.1)                                               | 91.9 (77.0–97.9)                                               | 100                                                            |
| NPV                 | 100 (91.7–100)                                        | 98.1 (88.8–99.9)                                               | 98.1 (88.8–99.9)                                               | 100                                                            |

TABLE 7 Diagnostic sensitivity and specificity estimates from a basic golden reference assay approach for SYBR green quantitative polymerase chain reaction (qPCR) assays Decapod Iridescent virus cell surface G (DIV CSG), papainase (PAP), patatin (PAT), and ATPase

<sup>a</sup>Gold standard model assumed that test A is perfect (100% sensitivity and 100% specificity; all patients with gold standard test positive are diseased and all patients with gold standard test negative are nondiseased). Values shown are estimated means with 95% confidence interval.

the LAMP assay (Gong et al., 2021) was reported at 190 copies per µl. The newly described RPA method (Chen et al., 2020) showed a sensitivity of 11 copies per reaction using a probit regression approach. No ASe data were presented for the nested PCR assays, and only a positive detection rate of the virus ranging from 15.2% in *Fenneropenaeus chinensis* (33 specimens) to 15.5% in *L. vannamei* (575 specimens) and 5 out of 10 specimens of *Macrobrachium rosenbergii* was reported (Qiu et al., 2017). The newly presented assays show a comfortable range of sensitivity compared to other published assays and could arguably be the method of choice when selecting a laboratory medium complexity-based approach that is cost-effective (SYBR real-time PCR) to monitor DIV1 (Tables 1 and 2).

In line with aforementioned studies, ASp has been assessed to be 100% in samples with a background of various shrimp pathogens, and no cross-reactivity has been detected for polychaete worm samples tested. Specificity has further been confirmed through direct amplicon sequencing of each qPCR amplicon for PAT, PAP, CSG, and ATPase and sequence authenticity annotated using the NCBI BLAST search tool confirming 100% sequence identity with the intended target (Table 5).

A population of 91 *L. vannamei* shrimp samples from Northern Asia-Pacific shrimp farms, suspected to be infected with DIV1, were analyzed for DIV1 infection status in this study, and the diagnostic performance of each assay was assessed using a golden standard reference

approach (Tables 6 and 7). All assays show good diagnostic performance with high sensitivity and specificity. In the differential comparison, qPCR assay DIV PAT reaches on average highest diagnostic sensitivity while maintaining a high level of specificity. On the contrary, Assay DIV ATPase showed highest specificity but a lower sensitivity. Overall, the presented assay performance is comparable to Qiu, Chen, Wan et al. (2018) who reported a DSe and DSp of 95.3 and 99.2%, respectively, for their TaqMan qPCR test from 323 DNA samples analyzed.

## 5. Conclusion

DIV1 is a critical emerging pathogen threat for the global shrimp industry, and all necessary steps need to be taken to avoid spread of the disease into the Asia-Pacific region and beyond. Hence, one key approach is to develop novel target gene and assays for this emerging pathogen and increase molecular tool capability for national and international biosecurity efforts. The novel targets and assays presented in this study are a vital step toward the tool expansion and will serve as a key foundation for increasing biosecurity preparedness. In particular, the DIV PAT and DIV PAP assays provide two new tools with high specificity and sensitivity toward monitoring and detecting this pathogen.

## ACKNOWLEDGMENTS

The authors would like to thank the Board of Genics Pty Ltd Australia for investing in core R&D to allow development of these essential tools to mitigate disease risk and increase biosecurity awareness of our global shrimp industry.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHOR CONTRIBUTIONS

Melony J. Sellars conceived and wrote the manuscript and organized funding. Louise Franz executed laboratory experiments and proof-read the manuscript. Ralf Joachim Moser conceived scientific experiments, executed laboratory work, undertook statistical analysis, and wrote the manuscript.

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## SUPER SHIELD EMS

# An active approach to prevent Early Mortality Syndrome/ acute hepatopancreatic necrosis disease (EMS/AHPND) in shrimp, is empirically proven effective in practice

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After intensive monitoring, aquaculture specialists from Grobest Vietnam and Can Tho University have had positive scientific evaluation on the Super Shield EMS product line of Grobest Vietnam's ability to maintain growth, enhance immunity, and proactively prevent shrimp against Early Mortality Syndrome (EMS).



A combination of Grobest's daily feeds and Super Shield EMS promotes healthy and well-grown shrimps

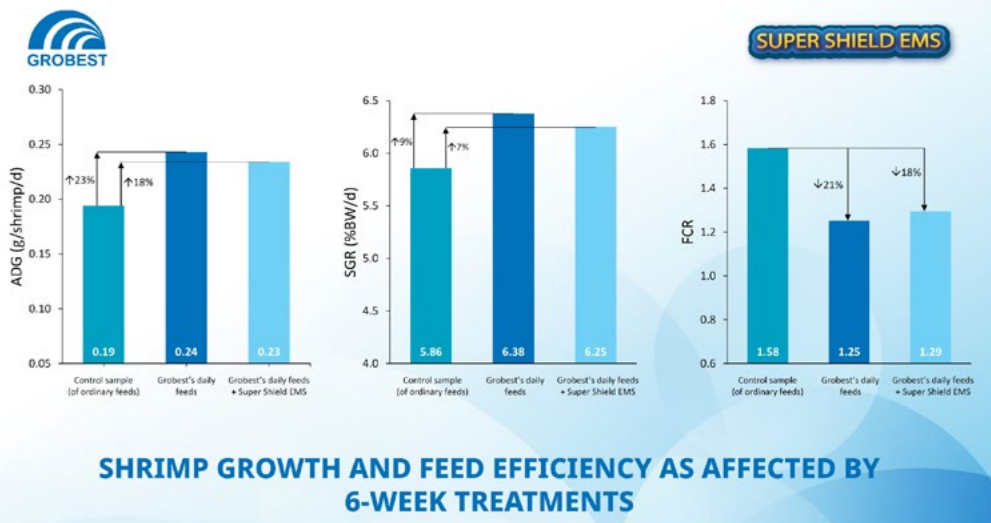
## A potential solution for preventing risks of EMS to Viet Nam shrimp farming

Can Tho University is a leading educational institution and technology transfer center in Vietnam, especially in aquaculture. In the past years, the university has gradually gained a reputation in assessing the quality of aquafeed, particularly shrimp feeds. This is the foundation for its collaboration with Grobest to conduct tests and experiments for the company's products, including the Super Shield EMS.

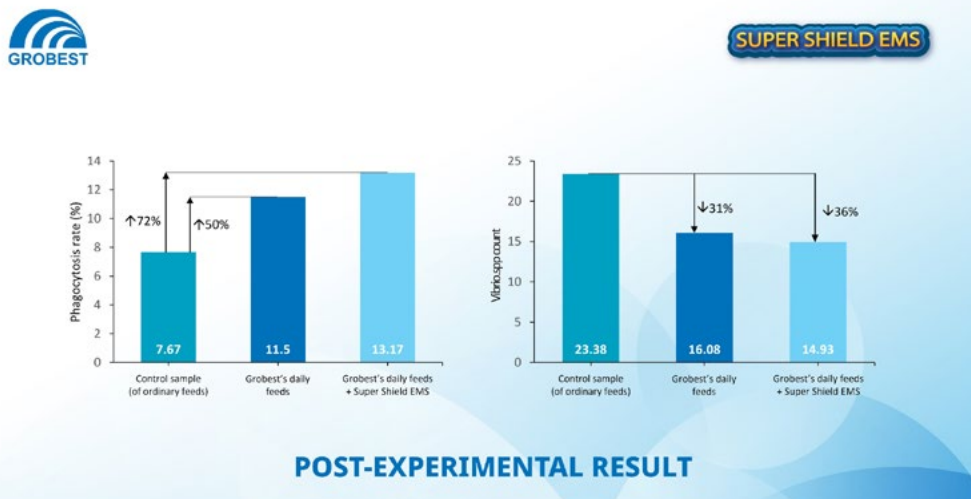
Grobest's Super Shield EMS has been tested by Can Tho University's Faculty of Aquaculture from July 22<sup>nd</sup>, 2021 to September 14<sup>th</sup>, 2021 with two phases: Growth phase (Six weeks) and challenge phase (Two weeks). Three treatments were compared: (1) Control feeds; (2) Grobest's daily feeds; (3) A combination of Grobest's daily feeds in the growth phase and Super Shield EMS **in the EMS challenge phase**. The third treatment is key to testing and determining the effectiveness of Super Shield EMS in preventing EMS in farmed shrimp.

Experts from Can Tho University's Faculty of Aquaculture listed the result of the experiments as follows: (1) Grobest's daily shrimp feeds promoted better growth and had better feed efficiency than the control feeds did; (2) The combination of Grobest's daily feed and Super Shield EMS was the best immunity boost for shrimps out of the treatments; (3) The post-infection survival rate of shrimp fed on the combination of Grobest's daily feed and Super Shield EMS was highest.

Therefore, it can be seen that Super Shield EMS is the new solution to help farmers proactively prevent the risk of EMS in shrimps and maximize their profits.



Super Shield EMS is the leading functional feed to help farmers proactively prevent - maximize profits against EMS



Post-experimental results

## The intensive, comprehensive, and objective monitoring procedure

To reach the above conclusions, experts from Can Tho University's Faculty of Aquaculture and Grobest have maintained scientific transparency when conducting the experiments: The samples were anonymously submitted to ensure objectivity and rigor of the result. The first goal of this experiment is to evaluate the growth performance of shrimps by comparing different treatments based on three indicators: **ADG (Average Daily Growth)**, **SGR (Specific Growth Rate)**, and **FCR (Feed Conversion Ratio)**. The second goal is to help all the stakeholders gain a closer look into the ability to promote shrimp's immunity, such as calculating the phagocytosis rate in shrimp's blood and the total of *Vibrio* spp. in the shrimp's gut, and EMS pathogens post-infection survival rate.

As mentioned above, three treatments were conducted: (1) A control feeds; (2) Grobest's daily feeds; (3) A combination of Grobest's daily feeds and Super Shield EMS during the experimental infection research. The results showed that **ADG** and **SGR** of the second

treatment is 23% and 9% higher than those of the first treatment respectively, while its **FCR** is 21% lower than that of the first treatment. The **ADG** and **SGR** of the third treatment is 18% and 7% higher than those of the first treatment respectively, whereas its **FCR** is 18% lower than that of the first treatment. Regarding the level of immunity, the phagocytic rate of shrimp fed on the combination of Grobest's daily feeds and Super Shield EMS was 72% higher than that of control feeds; additionally, the *Vibrio* load in the gut of shrimp fed on the combination of Grobest's daily feeds and Super Shield EMS was 36% lower than that of control feeds. Similarly, the figures in treatment (2) were 50% and 31% respectively compared to the control feed treatment.

In conclusion, thanks to the assessment based on rigorous experimental procedures carried out by Can Tho University's Faculty of Aquaculture, shrimp farmers can adopt the use of Grobest's daily feeds in the growthstage and Super Shield EMS when shrimps are susceptible to pathogenic bacteria or when in adverse climate conditions to bring out the best result

>> Super Shield EMS was officially launched by Grobest Vietnam on February 21, 2022. The product was researched and developed using strictly selected high-quality, high-efficiency, and nutrient-rich ingredients with exclusive additives developed by Grobest, such as **NutriRx™**, **NutriHepto™**, **XtraGo™**. Super Shield EMS has many traits of a good product, namely increase survival rate, growth rate, productivity, and profit of shrimp farming, especially in unusual weather conditions and the rapid escalation of the disease outbreaks.

for the shrimp's growth and immunity boost. This can be considered one of the achievements of Grobest Vietnam on its way of supporting the Vietnamese shrimp industry to 'grow stronger, grow faster, grow sustainably' and providing opportunities for farmers to reach the finish line with bumper harvests. ■



# The complex of agricultural high-technology DHN Gia Lai

On 14th May 2022, in Ia Le Commune, Chu Puh District, Gia Lai Province, the groundbreaking ceremony of the project “The Complex of Agricultural High-Technology DHN Gia Lai” took place.

Attending the groundbreaking ceremony, on behalf of the Ministry of Agriculture and Rural Development, there were the presence of Mr. Phung Duc Tien, Deputy Minister of Agriculture, together with leaders of the Ministry of Agriculture and Rural Development, leaders of the Provincial Party Committee, Gia Lai Provincial People's Committee, Provincial Leaders of the Central Highlands.

On the Dutch side, there was the presence of Mr. Daniël Stork - Consul General of the Kingdom of the Netherlands in Ho Chi Minh City; Mr. Co De Heus - CEO Royal De Heus; Mr. Koen De Heus - CEO Royal De Heus; Mr. Gabor Fluit - CEO De Heus Asia; Mr. Johan Van Den Ban - CEO De Heus Vietnam.

On the side of Hung Nhon Group, there was

Mr. Vu Manh Hung - Chairman of the Board of Directors, cum General Director of Hung Nhon Group;

Along with more than 750 guests are partners, customers, direct farms in the Central, Central Highlands, and Southeast regions, and employees of the two corporations.

The Complex of Agricultural High-Technology DHN Gia Lai” is in Ia Le Commune, Chu Puh District, with a total investment capital of about 1,030 billion VND. This is the 4th project in the DHN High-tech Agricultural Complex in Vietnam of two corporations, specializing in the production of high-quality agricultural products in a closed chain including: Selection and Production of Swine & Poultry Genetics; Automatic Slaughter Plant; produce animal feed in the direction of

organic; organic fertilizer production and trade in livestock products.

According to Chairman of Gia Lai Provincial People's Committee Vo Ngoc Thanh, Gia Lai's development strategy is to gradually shift from small and scattered livestock production to concentrated, industrial scale, high-tech application, organized closed production according to the value chain, forming a disease-free feeding area. Attracting large enterprises to invest in the livestock sector with 106 livestock projects applying high technology, total investment capital of over 15,000 billion VND.

Also on May 14, the Ministry of Agriculture and Rural Development signed a Memorandum of Understanding (MoU) on the Research and Development Program of the Feed Material

Area with AGRITERRA, a Dutch NGO, and De Heus Vietnam. Accordingly, the three parties will coordinate with local agencies and social organizations to build raw material production areas for the animal feed industry in the form of cooperatives. Focusing on cooperatives as the center and developing, thereby aiming at sustainable and effective agricultural production values, saving costs for farmers and increasing incomes for Vietnamese farmers.

Under the witness of Deputy Minister of Agriculture and Rural Development Phung Duc Tien, De Heus Group and Hung Nhon Group signed a MoU on a strategic cooperation program between the two groups. Accordingly, the two sides will jointly plan, build and develop projects in the field of animal husbandry and animal feed production. Specifically, from 2022 to 2030, De Heus and Hung Nhon will continue to cooperate to expand and develop a network of large-scale genetics projects of large-scale pig breeds and disease-free areas in 5 provinces. Highlands region. After completing the project in Gia Lai, it will next be deployed in Dak Nong and Kon Tum. The chain of projects, upon completion, will contribute to shaping the Central Highlands into a center for supplying piglets and developing leading hi-tech breeding models in Southeast Asia and Asia.

The goal for the genetics project of De Heus and Hung Nhon Group by 2030 will be: capacity of about 10,000 GGP, GP (equivalent to 80,000 gilts per year), pig herd capacity about 200,000 sows and about 6 million meat pigs. Total revenue is expected to be about 2 billion USD per year. With the field of chicken raising, De Heus and Hung Nhon built the Bel Ga breeding area in Tay Ninh. The Bel Ga project includes: 2 broiler chicken farms with a capacity of 25 million eggs/year, 250 safe broiler farms with a capacity of 25 million broiler chickens/year, and a complex of production plants and factories, processing high quality agricultural products in a closed chain.

#### **More information about the project:**

The DHN Gia Lai High-tech Agricultural Complex is expected to have a land use area of about 100 hectares, including farm of 2,500 pigs, selected and imported directly from the Netherlands, slaughtering pigs house, organic fertilizer factory, operation and support service area, organic farming area and green land, land for traffic and technical infrastructure, gathering area, purchasing area, preserve and pack fruits according to high quality export standards. Phase 1: The DHN Gia Lai hi-tech application breeding area has a total land area of 50 hectares, a capacity of 2,500 calves and grandparents and 25,000 gilts.

The complex is expected to apply 4.0 technology throughout the breeding process, helping to control livestock quality, optimize livestock performance, reduce product costs

### **Investor information:**

#### **Hung Nhon Group**

Hung Nhon Group is an enterprise that comes up from animal husbandry, with over 20 years of experience in development, has grown to become a “multi-industry” corporation operating strongly in many fields in Vietnam. Hung Nhon Group has special strengths in the field of large-scale livestock production, applying advanced technology with a closed process from breeding stock, feed, to processing and slaughtering according to ISO, Global GAP and ISO standards. other specialized international standards.

Hung Nhon Group currently owns 15 member companies and the DHN chain system, with 1,000 hectares of farms in the Southeast and Central Highlands, in which the broiler farm system provides the market with more than 3 million chickens. chickens per year, the laying hen farm system provides more than 130 million eggs per year, the system of swine genetics farms, GGPs, GPs, PS, and live pigs meets Vietnamese and international standards with total production of 14,000 breeding pigs and 375,000 commercial pigs per year.

#### **De Heus Corporation**

**De Heus Group (Netherlands)** is a corporation with more than 110 years of experience in providing innovative solutions for animal nutrition. De Heus currently has a scale of more than 100 modern factories worldwide, products are exported to more than 75 countries and territories worldwide. Particularly in Vietnam, De Heus owns 23 factories equipped with leading advanced automation technology lines and supervised production operation by international experts according to ISO 22000 & Global G.A.P standards.

De Heus Vietnam is proud to supply products to millions of households raising European quality livestock, poultry and aqua feed products, along with many breakthrough solutions to help farmers save costs, improving productivity and creating clean, safe and high-value products. De Heus Vietnam has pioneered and successfully cooperated with many partners to develop chain links in agricultural production in terms of high quality seed, clean meat production with traceability and qualified standards. exports, bringing many benefits to Vietnamese farmers and consumers.



and achieve high economic efficiency. Using a grid-connected solar power system, using 30% of clean electricity, reducing CO2 emissions by a large amount compared to using traditional power sources. Applying environmentally friendly and energy-saving measures in animal husbandry, organic farming, pig slaughter, and organic fertilizer production meeting Vietnamese standards and technical regulations and other organizations specialized international.

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# SUPER SHIELD EMS

## Proactive Protection

## Profit Maximization



EMS Prevention



Activate Immunity of Shrimp



Exclusive Additives: **NutriRx, NutriHepto, XtraGro**

**GROBEST VIET NAM**

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