global aquaculture

the advocate

The Global Magazine for Farmed Seafood

November/December 2015

the global aquaculture advocate

production

marketplace

On the cover:
Responsible aquaculture provides healthy food and important employment opportunities around the world. The Global Aquaculture Alliance has been proud to share this news through the *Global Aquaculture Advocate* magazine. Please continue to read the new *Advocate* online. Photo by Noppharat_th.

DEPARTMENTS

Page 20
Density Ups Feeding Response In Grouper
Contrary to common perceptions, grouper stocked at high density had greater feed intake and better feed conversion.

Page 66
Enhanced AHPND Detection
A study found that shrimp allowed to decompose prior to processing reflected improved PCR detection of AHPND.

innovation

GLOBAL AQUACULTURE ALLIANCE

The Global Aquaculture Alliance is an international non-profit, non-governmental association whose mission is to further environmentally responsible aquaculture to meet world food needs. Our members are producers, processors, marketers and retailers of seafood products worldwide. All aquaculturists in all sectors are welcome in the organization.

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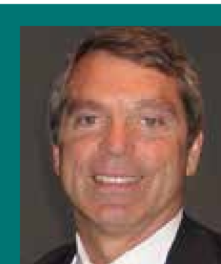
Advancement And Nostalgia



George Chamberlain, Ph.D.
President
Global Aquaculture Alliance
george.chamberlain@gaalliance.org

From the outset, the Global Aquaculture Alliance was convinced that aquaculture has enormous potential to grow and feed the world, provided it is practiced in a responsible way. During our brief history, many challenges have threatened to block this growth, but we have overcome these by working together. Aquaculture stakeholders around the world and throughout the value chain have found solutions to help avoid habitat destruction and improve social welfare, efficiency of resource use, food safety and accountability throughout the production chain.

We still have far to go on this journey, but we've learned that communication is a vital tool in the GAA mission to advocate, educate and demonstrate responsible aquaculture. We don't see our role in communication as reporting daily news events. We are more involved with recognizing trends, anticipating the challenges and working together with stakeholders to identify and describe potential solutions.

We have been greatly assisted in this process by the digital revolution in communications, from faxes to dial-up and now WiFi Internet access. From basic e-mail to text messaging on smart phones, Facebook, YouTube, Skype and a myriad of social media mechanisms. As the tools for communication have advanced, the *Global Aquaculture Advocate* has also adapted to serve your needs more efficiently.

The *Advocate* began in 1998 as a 16-page black and white newsletter with a circulation of a few hundred. In 1999, it became a magazine with partial color content, and in June 2005, it became a full-color magazine. Circulation gradually increased to a peak of 5,000 print copies per issue. In 2013, a searchable archive of all published articles was developed. This archive now includes thousands of articles. In 2010, a free digital *Advocate* was developed and offered to over 12,000 e-mail subscribers.

Now, we are pleased to announce that a web-based magazine format has been developed – see http://advocate.gaalliance.org. This will offer the content of the magazine in more digestible and timely chunks with an easily searchable platform. Previous articles will continue to be accessible through a search interface on the site, as well as complete digital issues back to 2010.

Change drives the *Advocate*, just as it drives the entire aquaculture industry. We are excited to embrace new approaches that will allow us to communicate more efficiently.

As you can see, the magazine has grown and evolved several times in its history to better serve you with authoritative information about global aquaculture issues, emerging technologies and opportunities to continuously improve production efficiency and sustainability. Change drives the *Global Aquaculture Advocate*, just as it drives the entire aquaculture industry. We are excited to embrace new approaches that will allow us to communicate more efficiently and conserve precious resources.

Nevertheless, it is with some nostalgia that we publish this final print issue of the *Advocate*. We would like to end with a review of some of the highlights of the last 17 years. And we may periodically publish a print edition of the *Advocate* to commemorate special occasions in the same way a handwritten letter still conveys a special meaning.

To all the contributors and readers of the *Advocate*, we extend a special thank you for your support over the years. We hope you continue to support our new web-based publication. As always, we look forward to your comments and suggestions.

Best regards,

George Chamberlain

George Chamberlain

One Journey Ends, Another Begins

Darryl E. Jory, Ph.D.
Editor, Development Manager
Global Aquaculture Advocate
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Incredible, truly quantum leaps have taken place in our industry in the last two decades, and the *Global Aquaculture Advocate* has been privileged to cover them for you during the last 17 years. In our special "Advocacy and Advances" section in this last regular print issue of the *Advocate*, we highlight some of the major developments made during our tenure.

Aquaculture continues to grow and currently contributes more than 50% of the world's seafood production. Annual global aquaculture output has more than doubled during these 17 years, from around 30 mmt in 1999 to close to 70 mmt today. Aquaculture plays a critical part in the global food security portfolio, and continued development will be essential.

According to a recent estimate by the international research organization WorldFish Center, by 2030, the world's population will require 232 mmt of seafood, approximately 62 mmt more than we are expected to produce unless significant steps are taken. Only our farmed seafood industry can provide this additional production.

Efficiency And More

How do we get there? I believe improving the efficiency of aquaculture production is the major strategy with the potentially largest impact. Ours is already a relatively efficient industry when compared to terrestrial livestock production. But we need to do much more.

Increasing sustainable production will require more output from established species; production development for new species; expansion into new inland, near- and offshore areas; improved domestication and genetic selection; better aquafeeds and new ingredients; improved health management; new production technologies with increased control; and better risk management.

We also need to better attract professional investors and accelerate the consolidation of the industry. And we cannot avoid considering the market, which will increasingly require more efficiency, quality control and traceability through the entire production chain. In other words, we need to become "industrialized" like other major meat-producing industries have done.

Many Thanks

As this era of the *Advocate* draws to a close, I would like to say thank you. Advertisers: Without your support, in particular, this magazine would have been just another idea. To the several hundred writers who contributed outstanding editorial content over the years, we are most grateful.

To our columnists and regular contributors – including Victoria Alday, Daniel Benetti, Eugenio Bortone, Kelly Coleman, Dave Conley, Daniel Fegan, Jose Fernandez, Tor-Eddie Fossbakk, Dick Gutting, Sue Heerin, David Kuhn, Tom Losordo, Graham Mair, Stephen Newman, Roy Palmer, Gary Rogers, Angel Rubio, Janice Schreiber, Wendy Spirduso, Tom Starkey, Albert Tacon, Ragnar Tveteras and Tom Zeigler – thanks for the great views and perspectives on so many topics. To Claude Boyd and George Flick, and the Urner Barry team of Paul Brown, Jr. – who were with us from the very beginning – we extend further thanks for your great support.

I am most grateful and honored for the opportunity to have worked with George and Susan Chamberlain during the humble beginnings of our magazine and the early years of GAA, when we could not have dreamed we would still be here in 2015 and beyond. Many other people also contributed to our success over the years, including Steve Hedlund, Cathy Herzig, Sally Krueger, Eric Pinon, Janet Vogel and Jane Walz.

The *Global Aquaculture Advocate* was largely George Chamberlain's vision, and without his constant and steadfast support and vision, as well as those of Wally Stevens, we would not be here today. Finally, to Lorraine Jennemann and David Wolfe, who make up the awesome production team of which I am a part, thanks, guys! See? It was worth it!

GAA and the *Advocate* – "feeding the world through responsible aquaculture" for over 17 years.

The Journey Continues

One of my favorite quotes, generally attributed to the immortal Ernest Hemingway, is "It is good to have an end to journey toward, but it is the journey that matters in the end." Well, this journey sure mattered, and we know we made a real difference.

The *Advocate* journey now continues by transitioning to a fully web-based format, which will allow us to keep providing content in support of GAA's goal to feed the world through responsible aquaculture. The *Advocate* will continue to focus attention on responsible aquaculture, on new technologies that improve production efficiency and sustainability, on market developments and trends, and many other relevant areas.

We have a great story to tell about our aquaculture industry, which has a major role to play in helping to produce the food needed to sustain our growing global population, and we must continue to tell that story.

It has been a privilege to serve you as the editor of the *Global Aquaculture Advocate* for 17 years, and I will continue supporting it on its new journey by contributing targeted editorial content.

Sincerely,

Daryl E. Jory

Darryl E. Jory

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Washington State China Relations Council
World Aquaculture Society
World Renderers Organization

Herzig Honored With GAA Achievement Award

Bill Herzig, a champion of seafood sustainability, has also supported the Global Aquaculture Alliance in many ways.

The Global Aquaculture Alliance has selected Bill Herzig as the recipient of GAA's Lifetime Achievement Award for 2015. Herzig was to accept the award during GAA's GOAL 2015 conference in Vancouver, British Columbia, Canada.

The career of this champion of seafood sustainability spans more than four decades. After graduating from the Georgia Institute of Technology, he took a job with Rich-SeaPak, working his way up to senior vice president of operations, procurement, quality assurance and commodity sales at the Georgia, USA, seafood company.

Herzig joined Florida, USA-based Darden Restaurants, the world's largest casual dining company, as director of seafood purchasing in 1997, graduating to vice president of seafood purchasing two years later. He rose to the position of senior vice president of purchasing and supply chain innovation. In this position, he was responsible for all purchasing across the 2,000-restaurant chain before transitioning to become president of Darden Aquafarms, an integrated lobster aquaculture facility in Malaysia.

Along with GAA President George Chamberlain, Herzig was instrumental in the formation of GAA in 1997. At the time, aquaculture – and particularly shrimp farming – was under attack from environmental interests spreading misinformation on mangrove deforestation. Stakeholders from 12 countries established GAA as a non-profit based upon a shared vision that aquaculture is vital to feed the world, but must grow in a coordinated and responsible fashion.

"This is the most satisfying thing that I have ever been involved in," long-time GAA board director Herzig said. "We did it because it was the right thing to do, and so many have joined the cause. As a result, the aquaculture industry now has the fundamentals in place to achieve the vision laid out three decades ago in the Blue Revolution."

"From the very beginning, Bill Herzig passionately and unselfishly devoted himself to GAA," Chamberlain said. "The organization would not exist today were it not for him.

"With his astute judgment, broad influence, business skills and leadership by example, he pulled us back from the brink of bankruptcy, guided us decisively through contentious issues and marshaled a strong network of long-term supporters. He worked his miracles quietly and unselfishly, like the silent hand of an angel."

"Bill Herzig and I have worked together in the seafood industry for more than 40 years," GAA Executive Director Wally Stevens said. "His forward thinking has led to innovation within every company and benefited the entire global seafood community."

Currently, Herzig is president of Sustainable Strategies and Initiatives. In addition to GAA, he has been heavily involved with the National Fisheries Institute.

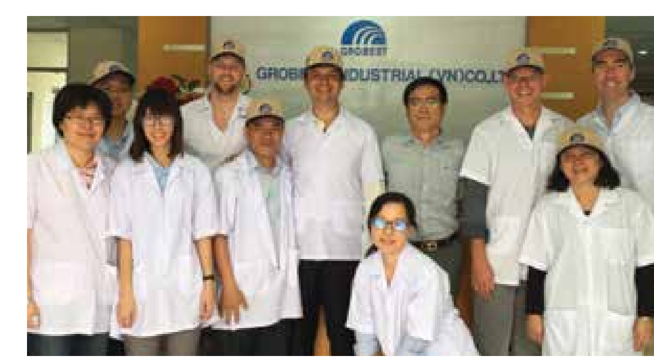
> **"From the very beginning, Bill Herzig passionately and unselfishly devoted himself to GAA. The organization would not exist today were it not for him."**
> **– George Chamberlain**

GAA, IDH Talk Collaboration With Grobest

Following the late-August Vietfish exhibition in Ho Chi Minh City, representatives from the Global Aquaculture Alliance and its Best Aquaculture Practices (BAP) division, and the Sustainable Trade Initiative (IDH) visited a Grobest feed mill in Vietnam to discuss ways to collaborate with key GAA supporters. GAA and IDH are working together to develop a partnership for supporting improvements in aquaculture.

"As we develop our new aquaculture strategy, market leaders like Grobest can really act as important enablers for the IDH mechanisms," said Aldin Hillbrands, aquaculture program director for IDH. "IDH will focus on the main development topics within feed, as well as broader farm-based issues such as disease. Grobest is ideally placed to support this focus, and we want to develop mutually beneficial cooperation with such companies."

Liu Yi Sung, general director for Grobest Vietnam, welcomed such cooperation to take on the challenges facing the aquaculture sector.



Representatives from GAA and the Sustainable Trade Initiative visited a Grobest feed mill in Vietnam to discuss ways to collaborate with key supporters.

The visit was arranged by GAA Development Director Iain Shone, BAP Vietnam representative Thanh Binh and Tracy Su of Grobest. Also in attendance was Flavio Corsin, Vietnam manager for IDH, and Roy van Daatselaar, program officer for IDH.

Mollusk Technical Committee Meets In Canada

Committee members toured oyster farms in this research vessel.

Members of the Best Aquaculture Practices (BAP) Mollusk Farm Technical Committee converged on the Deep Bay Marine Field Station of Vancouver Island University in British Columbia, Canada, in early October to hone the new BAP standards for mollusk farm certification prior to their projected release for public comment in November. The new standards will cover all major farmed mollusk species and eventually replace the current BAP mussel farm standards.

The committee spent two days working through the content of the standards in detail, but also took time to observe local oyster farms at close quarters in the field station's research vessel. Brian Kingzett, chairman of the technical committee, is also the manager of the Deep Bay station that hosted the meeting.

Kingzett was instrumental in ensuring that the committee membership represents the global scope of mollusk farming, both in terms of geography and species diversity. He has also brought to bear his understanding of the main issues confronting the shellfish industry in North America.

"It has been a real pleasure to work with such a committed group," Kingzett said. "Each brought a unique set of knowledge and skills, and this has been reflected in their input on the draft standards."

During the discussions, particular attention was paid to developing a workable approach for carrying capacity, with important additional expertise provided by Dr. Jon Grant of Dalhousie University, a member of the BAP Zone Management Committee.

Social Responsibility Specialist Joins SOC

Birgitte Krogh-Poulsen

Human rights expert Birgitte Krogh-Poulsen became the newest member of the Global Aquaculture Alliance Best Aquaculture Practices (BAP) Standards Oversight Committee (SOC) in September.

Krogh-Poulsen is an independent consultant with more than 15 years of experience in international development work in Africa and Asia, specializing in child protection; elimination of child labor, forced labor and human trafficking; and promotion of education and gender equality.

She previously worked as chief technical adviser for the International Labour Organization in Zambia and Thailand. Earlier, she worked for NIRAS Consulting Engineers.

Krogh-Poulsen filled a vacancy on the 12-member SOC, representing academia. The vacancy was left by Dr. Mudnakudu Nandeesha of India, who passed away in late 2012.

"To me, sustainable practices include environmental as well as social sustainability," Krogh-Poulsen said. "Decent labor conditions and upholding of rights are very much part of this agenda. I hope that my experiences will benefit GAA in its daily practice, and I look forward to an exciting new experience."

Krogh-Poulsen has bachelor's and master's degrees in political science from Århus University in Denmark. She resides in Viborg, Denmark.

BAP At Health, Food Safety Forum In Mexico

Best Aquaculture Practices (BAP) Manager of Supply Development Marcos Moya was among the speakers at the International Forum of Healthy Foods and Food Safety held in Aguascalientes, Mexico, in early September.

BAP was the only third-party aquaculture certification program invited to participate in the forum. Moya gave a presentation titled "Certificaciónes en Acuicultura."

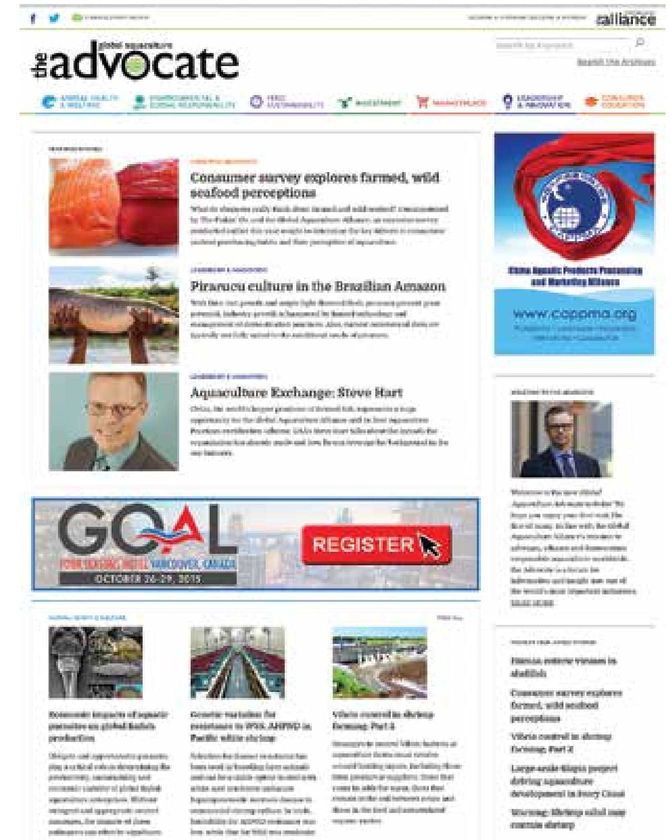
Attended by food safety experts from 22 Latin American countries, the conference was organized by Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria and Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria to update and educate the bodies' supervisors and hundreds of auditors on food safety trends and new methodologies.

SENASICA is Mexico's National Service for Animal Health, Food Safety and Quality. SAGARPA is the Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food.

Addressed at the forum were topics such as international health regulations, regional food safety initiatives, risk analysis, sampling and diagnosis, and national toxic waste monitoring and control. In addition to aquaculture, the program addressed the beef, pork, poultry and produce sectors.

Advocate Transitions To New Website

Editor Wright: 'It's Your Site'



Material on the *Advocate* website is organized within searchable categories that align with the major challenges facing aquaculture growth.

With the October 1 launch of the new http://advocate.gaalliance.org website, the *Global Aquaculture Advocate* continued its transition toward an online-only publication. This November/December 2015 issue is the last printed edition of the *Advocate*.

Since 1998, the *Global Aquaculture Advocate* has been integral to GAA's identity as a trusted source of information on responsible aquaculture. The new *Advocate* blends the best of the print magazine with the convenience and interactivity of a website.

"The magazine provided reliable and highly technical content about aquaculture production and innovation," new *Advocate* Editor James Wright said. "We'll still be highlighting the research work the magazine has always been known for, but we'll be pairing it with supply-, production- and market-related news about aquaculture and its myriad products."

The new material on the *Advocate* website is organized within searchable categories that align with the major challenges facing aquaculture growth – from environmental and social responsibility to animal health, investment and the evolving seafood marketplace.

Longtime *Advocate* Editor Darryl Jory, who is remaining on staff as editor emeritus, will continue to contribute. The site will also feature an archive section that includes downloadable files for each issue of the *Advocate* from 2010 onward, select editions in Spanish and a Google-based search system for articles back to 2000.

The website is accompanied by a weekly newsletter that will contain some of the latest *Advocate* articles and select GAA and Best Aquaculture Practices news and activities.

Access to the site will be unlimited through December 31. As of January 1, 2016, users who register will be able to read up to five *Advocate* articles, and only those with a new "subscriber" or higher-level membership to GAA will have unlimited access to the website.

"Please bear with us as we build the site into what we hope it will become: a one-stop destination for news and insights into how the aquaculture industry is moving forward," Wright said. "We will be relying on our readers to guide us in the future regarding editorial direction and the overall look and feel of the site. It's your industry. It's your voice. It's your site."

BAP Site Updates Highlight Program Integrity

Look for the new program integrity section of the BAP website.

The Global Aquaculture Alliance's Best Aquaculture Practices division has updated its website with new content that helps explain the lengths to which BAP goes to ensure program integrity. The information and documents are categorized under the new "Program Integrity" tab on the main navigation bar in the upper right of the BAP home page at http://bap.gaalliance.org.

A downloadable "BAP Program Integrity FAQs" document clarifies the various roles, qualifications and procedures for program integrity regarding BAP, certification bodies, auditors and facilities.

The "BAP Multi-Star Integrity Tips for Buyers" file is designed to assist seafood buyers who purchase seafood produced under the BAP certification program. It can help buyers evaluate the validity of two-, three- and four-star BAP products. The document can also clarify misperceptions about multi-star status. For example, BAP certification of facilities means they are capable of producing seafood bearing BAP stars, not that 100% of their production meets the requirements.

A "Logo Usage, Brand Identity and Product Claims" page has been added to the site. It includes guidelines designed to ensure the BAP logo and marks are used appropriately and that any claims associated with BAP are portrayed in an accurate manner. There are separate guidelines for production facilities, marketing and communications, and retail and foodservice purposes.

Farms Lead Recent BAP Certification Growth

The global expansion of Best Aquaculture Practices (BAP) certification continues, with dozens of additional facilities certified in recent months (Table 1). Multiple key culture species from salmon and shrimp to trout and carp are produced at the facilities.

Farms were the top category for BAP growth, with new facilities certified in Asia, South America and North America. New seafood processing plants also came on board, along with hatcheries from Canada to Australia and several feed mills.

Chile's **Multiexport Foods S.A.** is now eligible to offer three-star BAP salmon. Three-star status denotes that seafood originated from a BAP-certified processing plant, farm and feed mill or hatchery.

Multiexport's Puerto Montt processing plant and eight of its farm sites in Chile's Patagonia region are BAP-certified. All of the feed mills from which Multiexport sources feed are also certified under the BAP program.

"These types of accomplishments only occur when there is a sincere commitment from the entire organization," said Francisco Lobos, Multiexport's environmental manager. "Sustainability and responsible farming is an integral part of our culture."

Petuna Aquaculture Pty. Ltd.'s Atlantic salmon and ocean trout hatchery in East Devonport, Tasmania, recently became the world's first multispecies hatchery to attain BAP certification. It joined Petuna's salmon and ocean trout farm sites, which achieved certification earlier this year, in the accomplishment.

Petuna produces whole fish, fillets and hot- and cold-smoked salmon and trout products that it markets domestically and in Asia, the United States and the United Arab Emirates.

"We take our social and environmental license very seriously," said Mark Porter, CEO of Petuna Aquaculture. "BAP certification across our production cycle assures our customers and the people of Tasmania that Petuna will always strive to deliver the best product possible, in a sustainable manner that meets their expectations on every occasion."

Congratulations to **Gisis**, Ecuador's first feed mill to attain BAP certification. This shrimp and tilapia feed mill is located in Durán, Ecuador.

Gisis is part of the Nutreco Group, a global leader in animal nutrition and fish feed. The company's advanced feed solutions are at the origin of food for millions of consumers worldwide.

Congratulations also go to **Vitapro S.A.,** whose shrimp feed mill in Trujillo, Peru, became the country's first feed mill to attain Best Aquaculture Practices certification. Vitapro's Inbalnor S.A. feed mill in Ecuador attained BAP certification in August. Both produce shrimp and fish feed under the Nicovita brand.

Table1. Recent BAP certifications around the world.

Facility	Country	Species
Farms		
Caseamex Hatchery and Technical Fishery Center	Vietnam	*Pangasius*
Cau Mau Seafood Processing and Service Joint Stock Co. – Dam Doi Farm	Vietnam	Shrimp
Exportadora Los Fiordos Ltda. (3 Farms)	Chile	Salmon
Fuqing Huawang Agriculture Comprehensive Development Co., Ltd.	China	Shrimp
Granja Arco Azul Truchas Belmira SAS	Colombia	Rainbow Trout
Guangdong Yujia Seafood Co., Ltd. – Huazhou Beizhuang Reservoir	China	Tilapia
Hainan Shenghaiyuan Agricultural Scientific and Technological Development Co., Ltd. – Jinlian Base	China	Tilapia
Hainan Shenghaiyuan Agricultural Scientific and Technological Development Co., Ltd. – Longma Base	China	Tilapia
Hainan Xinhaiyang Aquatic Products Co., Ltd.	China	Tilapia
Jagadeesh Marine Exports – IOM 1 – Thota Sita Rama Lakshmi	India	Shrimp
Jagadeesh Marine Exports – IOM 2 – Mantena Venkata Satya Bapiraju	India	Shrimp
Jagadeesh Marine Exports – IOM 3 – Krovidi Nagendra Prasad	India	Shrimp
Kelly Cove Salmon Ltd. – New Brunswick (2 Farms)	Canada	Salmon
Liang Weitao Aquaculture Farms	China	Tilapia, Carp, Dac, Bighead
Pescados Frescos do Colombia S.A.	Colombia	Rainbow Trout
Phu Thanh Co., Ltd.	Vietnam	Shrimp
Seafresh IOM 2 (5 Farms)	Thailand	Shrimp
Seafresh IOM 3 (4 Farms)	Thailand	Shrimp
Seaprimexco Vietnam IOM 1	Vietnam	Shrimp
Tan Thuan Dong Farm	Vietnam	*Pangasius*
Thai Royal Frozen Food Co., Ltd. – Khachonvot Farm	Thailand	Shrimp
Thai Royal Frozen Food Co., Ltd. – Nakornpong Farm	Thailand	Shrimp
Thai Royal Frozen Food Co., Ltd. – Sawee Farm 1	Thailand	Shrimp
Thai Royal Frozen Food Co., Ltd. – S. Sununtha Farm	Thailand	Shrimp
Thai Royal Frozen Food Co., Ltd. – Thaveepong Farm	Thailand	Shrimp
Thai Royal Frozen Food Co., Ltd. – Thaveepong Farm 2	Thailand	Shrimp
Xuwen County Xinliao Shidaoxiao Farm	China	Shrimp
Yangxi County Shunxing Sea Fishery Co. Ltd. – Maodong Reservoir Farm	China	Tilapia
Hatcheries		
C.P. Vietnam Corp. – Branch Bac Lieu	Vietnam	Shrimp
Cressy Hatchery	Australia	Salmon, Trout
Gold River Hatchery	Canada	Salmon
Guangdong Fishery Germplasm Protection Organization	China	Tilapia
Jay Kay Hatchery II	India	Shrimp
MAS Aquatechniks Private Ltd.	India	Shrimp
M/S Hatcheries Private Ltd.	India	Shrimp
MSK Hatcheries	India	Shrimp
Oceanic Edibles International Ltd.	India	Shrimp
Srinidhi Biotechnologies	India	Shrimp
Processing Plants		
Browns Bay Packing Co., Ltd.	Canada	Salmon
Can Tho Import Export Seafood Joint Stock Co.	Vietnam	*Pangasius*
K. F. Foods Co., Ltd.	India	Shrimp
Mangala Seafoods	India	Shrimp
Pescados Frescos de Colombia S.A.	Colombia	Rainbow Trout
P.T. Wahyu Pradana Binamulia	Indonesia	Shrimp
R. F. Exports	India	Shrimp
Sanchita Frozen Foods Private, Ltd.	India	Shrimp
Sharat Industries Ltd.	India	Shrimp
Feed Mills		
Elite Bio Feed Co., Ltd.	China	
Fujian Haid Feed Co., Ltd.	China	
Goazhou City Pans Feed Co., Ltd.	China	
Grobest Feeds Corp. Ltd.	India	
Inbalnor S.A.	Ecuador	

Photo by Steve Winter.

Global Aquaculture Advocate

17 Years of Aquaculture Advances and Advocacy

Shortly after the Global Aquaculture Alliance formed in 1997, the *Global Aquaculture Advocate* became its primary voice, sharing the important news of responsible aquaculture with GAA members and the greater seafood community. The look of the publication changed as it evolved from a basic newsletter to a full-color magazine, but the core message didn't. As aquaculture continues to supply wholesome and varied seafood products to the world's growing population, it must do so in increasingly efficient and sustainable ways.

Some of the many advances in aquaculture covered in past editions of the *Advocate* are illustrated in the following pages. As the *Advocate* moves to an online-only format after this final print issue, it will continue to present information and insight into one of the world's most important and fastest-growing industries – aquaculture.

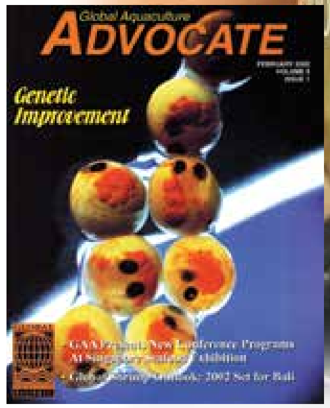
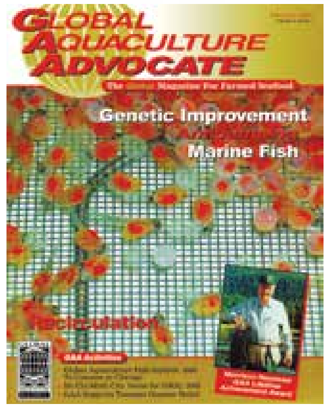


Photo by Darryl Jory.



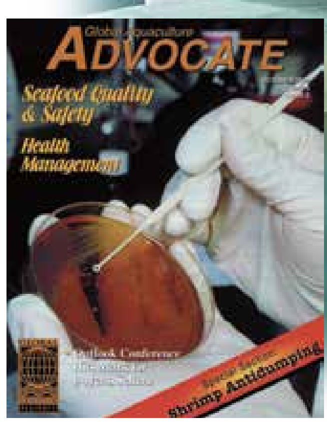
Selective Breeding

Through the controlled mating of animals to produce offspring with desirable traits, selective breeding or genetic improvement has increased production efficiency and improved product characteristics for a range of aquaculture species.

In general, selective breeding of aquatic species can typically result in growth gains of 10% or more per generation. Genetic improvement of tilapia has led to morphologically larger fish with higher meat yields. Selected resistance to disease has multipled survival for animals in disease-affected areas. Thanks to selective breeding, fish and shrimp can now be raised under a broader range of temperature and salinity.

Genetic improvement is a factor that underlies success across the aquaculture process. Despite selective breeding's great potential, only an estimated 10 to 12% of global aquaculture production is currently based on genetically enhanced stocks. New methods and technologies can help support wider application of selective breeding.

Photo by Stephen Ausmus, USDA-ARS.



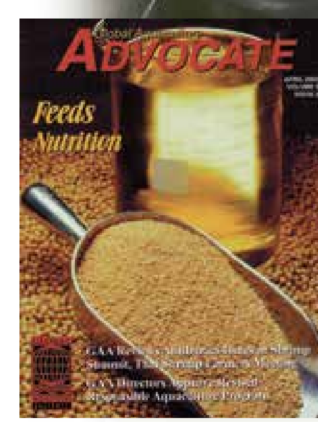
Health Management

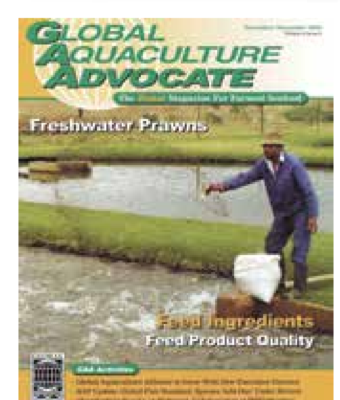
Losses to diseases caused by viruses, bacteria, parasites and other pathogens have cost the aquaculture industry billions of dollars. Fortunately, many tools have been developed to diagnose and treat culture animals with disease issues. Extensive research has led to a variety of reliable, fast and highly sensitive diagnostic tests.

DNA-based immunoassays and diagnostic methods including polymerase chain reaction amplification, in situ and dot blot hybridization, enzyme-linked immunosorbent assays and radioimmunoassays are faster and cheaper than traditional methods. On the management side, better understanding of host-pathogen interactions led to biosecurity measures that effectively exclude pathogens from culture facilities.

The production of specific pathogen-free and specific pathogen-resistant stocks in species like penaeid shrimp has also advanced health management, along with the development of fish vaccines and feed additives that strengthen animals' immune systems and reduce susceptibility to some diseases.

Photo by George Chamberlain.



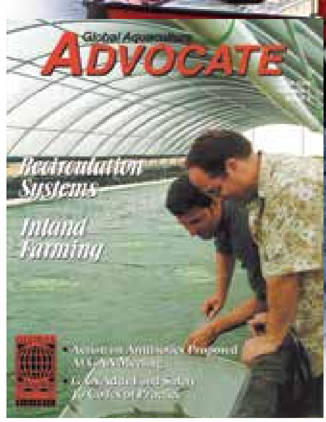


Nutrition and Feeds

Expanded aquafeed production has been crucial for the growth of aquaculture. Nutritionist Dr. Albert Tacon reported that to meet projected demand, annual global aquafeed production must rise from 35 mmt in 2010 to 70 mmt by 2020.

Researchers have gained great understanding of the nutritional needs of varied farmed species under culture conditions, including quantitative requirements for macro- and micronutrients such as the essential fatty acids needed for bodily functions. More-functional and environmentally oriented aquafeeds are the result.

There has been a clear shift from the widespread use of ingredients like fishmeal and other aquatic animal meals and lipids to nutrients derived from agricultural activities on land. Looking forward, there is a critical need for additional novel feed ingredients and nutrients, as well as new manufacturing technologies and better aquafeed management that delivers to culture animals high-quality feed in the proper amounts and at the right times and locations.



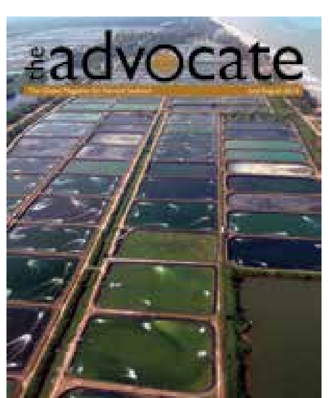


Photo courtesy of Norwegian Seafood Export Council.

Production Systems

Hatchery and nursery systems have improved tremendously over the last two decades. Tanks and raceways upgraded with better water quality monitoring and treatment, expanded aeration and tighter biosecurity. In parallel came improvements in broodstock management and conditioning, as well as multiphase larval management and feeding techniques incorporating live feed replacements and microbial enhancements.

Land-based growout systems have benefitted much from technological advances. Many earthen ponds are now deeper and lined with plastic materials that allow high rates of aeration and efficient sludge removal. Extensive biosecurity measures prevent the introduction of pathogens. Biofloc technology has become integral to many tank- and raceway-based recirculating aquaculture systems with reduced water exchange. Harvesting has been mechanized to a large extent.

Near-shore and offshore cage production technology has also enjoyed tremendous sophistication. Newer cage designs, both surface and submerged, feature improved anchoring systems, mechanized feeding and automated monitoring.

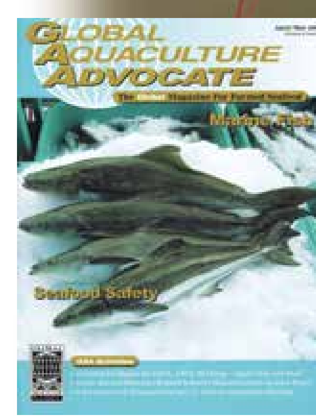


Photo courtesy of Nicovita.

Big Four, Emerging Species

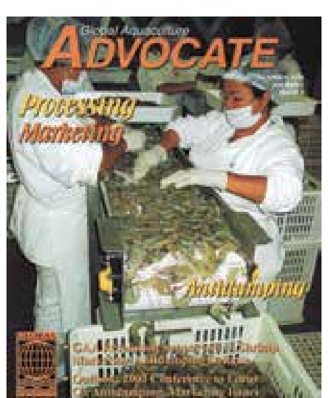
Over 250 aquaculture species are produced around the world. Four, in particular, experienced extraordinary growth in the last two decades.

Tilapia are the second most-produced fish in the world. Breakthrough hormonal sex reversal allowed monosex populations raised to uniform, marketable sizes. White shrimp is the next most widely cultivated aquatic species. Selective breeding created fast-growing, disease-resistant shrimp now used widely in Asia and the Western Hemisphere.

Sea cage culture of Atlantic salmon introduced the high-value species to various regions. Almost all production uses cages with initial land-based smolt production. Selective breeding produced hybrid strains that bolster the industry. *Pangasius* aquaculture that began in Vietnam has expanded elsewhere. Annual production in earthen ponds and cages exploded in the mid-2000s, with wide exportation of *Pangasius* products.

Emerging species – including marine fish and various Amazonian freshwater fish – will undoubtedly contribute to the expansion of the aquaculture industry.

Photo by Gail Hannagan.



Globalization and Integration

Seafood is among the most traded food commodities worldwide. A significant share of total seafood production (including farmed output) is exported in raw, frozen or value-added forms. Global seafood exports increased from about 25% in 1976 to over 40% currently, with 197 countries involved.

Sustained demand, trade liberalization policies, globalization of food systems and technological innovations have furthered the overall increase in international fish trade. Improvements in processing, packaging and transportation, as well as changes in distribution and marketing have fueled the shift from local consumption to international markets.

Vertical integration and consolidation in which companies completely control seafood from the first stages of production through marketing is a growing trend for species like salmon, shrimp and tilapia. In the salmon industry, for example, the top 25 companies control almost 75% of the total global production.

Certification

Consumers increasingly care about what they eat, how their food is produced and the impacts food production has on the environment and society. Especially as aquaculture production rises to meet growing demand, certification programs help consumers make good seafood choices.

Various private entities certify aquaculture operations against standards of best practices. The companies can then differentiate their products using eco-labels. Producers are rewarded for their practices through increased demand and preferred status with suppliers.

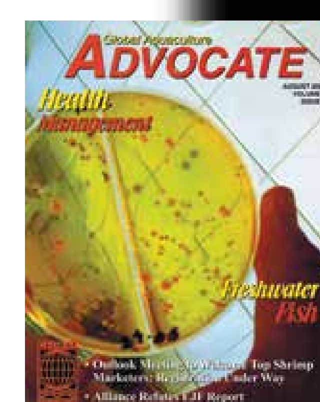
GAA's Best Aquaculture Practices (BAP) program is the leading international, third-party certification system for the entire aquaculture production chain. BAP certification of facilities verifies that fish and crustaceans are produced using environmentally and socially responsible processes. The BAP certification standards address every key element of responsible aquaculture, including food safety.

Biotechnology

New fields of biological study – such as genomics, metabolomics and proteomics – have emerged as important biotechnology tools in aquaculture. Work in these fields involves the study of very large data sets and emphasizes the quantitative analysis of all components in a biological system.

For example, genomic tools can identify genetic markers associated with commercially important traits in animals and explain the molecular basis of many physiological processes. Nutrigenomics allows the development and refinement of natural and bioactive compounds that provide health benefits, including the accurate targeting of specific diseases.

Microbial technology involving pre- and probiotics is another important area. As alternatives to antimicrobials, probiotic bacteria are applied as microbial control agents in disease control.



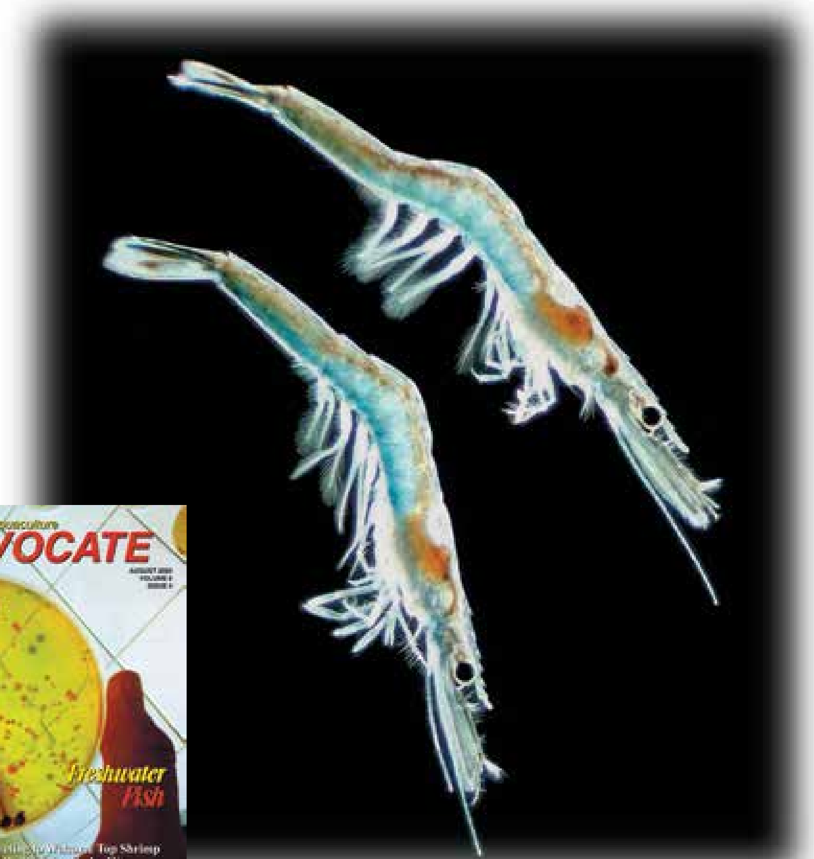


Photo by Peter Bridson.

Parasite Treatment Reduces *Flavobacterium Columnare* Infection In Tilapia

The authors treated *Trichodina* with formalin in tilapia and determined fish survival following a bacterial challenge with *F. columnare*.

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Summary:
The authors conducted a study to evaluate whether treatment of *Trichodina*-parasitized tilapia with formalin would improve fish survival and reduce *F. columnare* infection. Tilapia not treated with formalin showed significantly higher mortality than treated fish. The untreated fish also had significantly higher numbers of *F. columnare* in gill, kidney and liver tissue than in treated tilapia. The bacterial load of untreated fish was 12-fold higher than that of fish treated once with formalin and 39-fold higher than the load in fish treated twice.

Trichodina are ciliated protozoan parasites of fish commonly found on the skin and gills. Fry and fingerling fish are especially susceptible to parasitism by *Trichodina*. *Trichodina* causes irritation by feeding on the epithelial layers of cells covering the surfaces of the skin and gills of the fish. *Trichodina* can cause serious pathological changes and mortalities among heavily parasitized fish. High numbers of *Trichodina* on fish can result in abrasion with the development of lesions and ulcers that allow secondary bacterial infections to develop.

Flavobacterium columnare, a Gram-negative rod bacterium, is the causative agent of columnaris disease. It frequently causes high mortality in many commercially important freshwater fish worldwide, including tilapia. Columnaris affects all life stages, from newly hatched fry to fish that have reached market size. Columnaris is generally regarded as an external infection of fish with clinical signs of skin lesions, fin erosion and gill necrosis.

Concurrent Disease

Parasites and bacteria are common inhabitants in water at fish farms. Studies of parasite-bacteria interactions have showed that concurrent infections increase the severity of some infectious diseases, especially bacterial diseases.

In a previous report, tilapia infected with high numbers of *Trichodina* at a recirculation production facility had skin injuries that led to bacterial streptococcosis and edwarsiellosis that could not be controlled by antibiotics. Control of the parasite with formalin resulted in a decrease in overall deaths.

Treatment Study

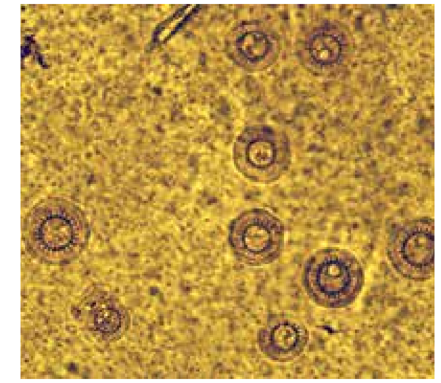
Building on this, the authors performed a study to evaluate whether treatment of *Trichodina*-parasitized tilapia with formalin would reduce *F. columnare* infection in fish and ultimately result in improved fish survival.

Sex-reversed hybrid tilapia of about 10-cm length reared in three, 340-L indoor tanks at the Aquatic Animal Health Research Unit of the U.S. Department of Agriculture's Agricultural Research Service in Auburn, Alabama, USA, were found to be parasitized with *Trichodina* on both skin and gills. The infection intensity was measured at 4 to 8 *Trichodina* within a single 100-time magnification view under a microscope.

Flavobacterium columnare was isolated from a diseased channel catfish from Alabama. The isolate was inoculated in broth and incubated aerobically at 28° C. Following 24 hours of growth, the concentration of the bacterium was determined by bacterial plate count.

Parasite Treatment

A total of 390 hybrid tilapia were divided into three groups. One group received no parasite treatment. The second group was treated in a single water bath with 150 mg/L formalin for one hour. The third was treated in a water bath with 150 mg/L formalin for one hour on two consecutive days. The fish were allowed to recover for five days after the treatments.



This magnified wet-mount sample shows *Trichodina* infection on tilapia caudal fin tissue.

Ten fish were sampled from each group to enumerate their parasite loads and verify the fish were not infected with *F. columnare* using real-time polymerase chain reaction (qPCR). The fish were then divided into 18 tanks at 20 fish/tank for *F. columnare* challenge.

F. columnare Challenge

Each group of fish from the formalin treatment phase was stocked in triplicate tanks. Half of each group was challenged via immersion in buckets of water with *F. columnare* at a concentration of 3.2 x 10^7 colony-forming units (CFU)/mL for 15 minutes. The remaining fish were not exposed to the bacteria, but kept in water with the same amount of Shieh broth for the same duration.

Two fish were randomly sampled from each tank one and three days after the *F. columnare* exposure. To evaluate *Trichodina* infection, wet-mount samples of skin and gill filaments were observed under a microscope.

Gill, liver and kidney tissues of about 20 mg were then collected from two fish and macerated in a microcentrifuge tube for *F. columnare* quantification. DNA was extracted and purified using standard methods. qPCR testing was used to quantify the *F. columnare* in infected fish.

Results

Prior to the *F. columnare* challenge, no *Trichodina* was observed five days post parasite treatment on the skin and gills of fish that were treated twice with formalin (Table 1). The single formalin treatment significantly reduced parasite prevalence and intensity as compared to the untreated fish. All non-treated fish were infected by *Trichodina* at an intensity of 4-6 parasites/viewing area.

After exposure to *F. columnare*, the untreated tilapia parasitized by *Trichodina* showed 37.5% mortality, while mortality for those treated with formalin was 16.7% or less (Table 2). Tilapia treated twice experienced 6.4% mortality. No mortality was observed in the parasitized tilapia that were not challenged with *F. columnare.*

One day after exposure to *F. columnare*, the parasitized fish without treatment showed significantly higher numbers of *F. columnare* in their gills compared to those treated with formalin (Table 3). The bacterial load was 27,075 genome copies (G.C.)/mg in the gills of parasitized fish without treatment – 12-fold higher than those treated once with formalin (2,250 G.C./mg) or 39-fold higher than those treated twice with formalin (699 G.C./mg).

The parasitized fish without treatment also showed higher bacterial numbers in kidney and liver tissue than those treated with formalin, with concentrations of 207 and 266 G.C./mg, respectively.

Fish treated once with formalin had bacterial concentrations of 88 G.C./mg in kidney tissue and 127 G.C./mg in liver tissue. No *F. columnare* was detected in kidney and liver of fish treated twice.

Table 1. Infection prevalence and intensity of *Trichodina* in skin of tilapia prior to and one day after bacterial challenge.

Number Of Treatments	Number Of Fish	Infection Prevalence (%)	Infection Intensity
Prior to *F. columnare* Challenge			
0	30	100	6.4
1	30	20	0.3
2	30	0	0
One Day After *F. columnare* Challenge			
0	18	100	3.7
1	18	22	0.2
2	18	0	0

Infection intensity = Number of *Trichodina* on fish skin or gill per viewing area under a microscope.

Table 2. Cumulative mortality of tilapia with parasite treatment after challenge by *F. columnare*.

Number Of Treatments	*F. columnare* Challenge	Number Of Fish	Number Of Dead	Mortality (%)
0	Yes	48	18	37.5
1	Yes	48	8	16.7
2	Yes	48	3	6.3
0	No	48	0	0
1	No	48	0	0
2	No	48	0	0

Table 3. Genome copies of *F. columnare* in tissues of tilapia with parasite treatment one day after exposure to *F. columnare*.

Tissue	Treatment	G.C./mg
Gill	0	27,075
	1	2,250
	2	699
Kidney	0	207
	1	88
	2	0
Liver	0	266
	1	127
	2	0

Perspectives

Trichodina are transmitted by fish-to-fish contact and/or contaminated water. Improving water quality could be effective in reducing parasite infections in farmed fish. When fish parasites are detected in pond fish, treatment may be considered if the parasites are treatable, and treatments are economically feasible. Early detection and treatment may prevent heavy parasite loads on fish and limit the spread of parasites to fish in other tanks or ponds.

Parasite infections can disrupt the first line of defense in the skin and gills of fish, thereby creating portals of entry for bacterial pathogens. The formalin treatment of *Trichodina*-parasitized fish reduced bacterial invasion and ultimately improved fish survival.

This work suggested that prevention and treatment of parasite infections in fish are important parts of fish health management that not only reduce the direct damage caused by parasites, but also reduce fish mortality due to secondary bacterial infection.

Provided that optimum water quality parameters are maintained, white grouper can be a suitable candidate for intensive farming systems.

Increased Density Improves Feeding Response, Growth Performance In Grouper

Summary:
White grouper is a promising candidate for intensive aquaculture because of its high market value, excellent taste and rapid growth. A growth trial using hatchery-reared grouper was carried out to study the effects of stocking density on feed intake and subsequent growth. Contrary to common perceptions, fish stocked at the highest density had higher feed intake and body weights. Improved feed conversion was also achieved with the increased feed intake, which may have been a behavioral response to crowding in the culture tanks.

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White grouper, *Epinephelus aeneus*, is a Mediterranean fish species that like many Asian groupers is a very promising candidate for intensive aquaculture because of its high market value, excellent taste and rapid growth. Under domestication at the National Centre for Mariculture in Eilat, Israel, white grouper has been successfully reproduced in captivity, and a formulated feed had been developed for its culture.

One of the major steps in this development is to define the potential of the fish for growth under optimal conditions and how this growth is affected by the quality and quantity of the feed the fish eat. In addition, other factors that influence growth, such as feeding behavior and water temperature, should be determined.

Table 1. Formulation and proximate analysis of grouper feed.

Ingredient Content	
Fishmeal (g/kg)	740
Corn starch (g/kg)	200
Fish oil (g/kg)	50
Vitamin mix (g/kg)	10
Analysis (as fed)	
Dry matter (g/kg)	919
Crude protein (g/kg)	498
Crude lipid (g/kg)	119
Ash (g/kg)	114
Gross energy (MJ/kg)	19.95

Table 2. Performance parameters of white grouper at increasing stocking densities.

Number of Fish in 200 L	Final Weight (g)	Stocking Density (kg/m³)	Feed Intake (%/biomass/day)	Feed-Conversion Ratio
20	25.52	2.5	2.68	0.95
40	34.62	6.9	2.90	0.80
60	43.24	13.0	3.06	0.80
80	45.59	18.2	3.17	0.77
100	50.46	25.2	3.48	0.77

Table 3. Body composition of white grouper raised at increasing stocking densities (wet weight).

Number of Fish in 200 L	Dry Matter (g/kg)	Protein (g/kg)	Lipid (g/kg)	Ash (g/kg)	Energy (MJ/kg)
Initial	240	164	33	39	5.078
20	257	167	50	41	5.703
40	274	178	58	43	6.120
60	274	173	61	41	6.189
80	268	170	59	41	6.097
100	270	169	64	39	6.218

As part of the research in these areas, a growth trial using hatchery-reared grouper was carried out by the author to study the effects of stocking density on feed intake and subsequent growth.

Study Setup

Duplicate groups of white grouper with initial weights of 5.2 g were reared for 63 days at stocking densities of 20, 40, 60, 80 and 100 fish/200-L tanks. The average water temperature was 23.3° C, and salinity was 40 ppt. Feed was formulated mainly from fishmeal and fish oil and steam pelleted to a size of 1.6 mm (Table 1).

The grouper were fed manually twice a day to apparent satiation, and feed intake was recorded daily. Feeding ceased once pellets were found remaining on the bottoms of the tank for more than a minute.

Special attention was also paid to maintain water quality parameters with increasing biomass via increased water flow and sufficient aeration. The tanks were also cleaned daily of any waste products.

Results

At the end of the trial, the fish grown at the highest density reached a final weight of 50.5 g, compared to 25.5 g for fish kept at the lowest density. This corresponded to stocking densities of 25.2 kg/m³ and 2.5 kg/m³, respectively. The improved growth was obviously due to higher daily feed intake, which increased gradually from 2.7 to 3.5% of biomass for fish kept at the low and high densities, respectively (Table 2). Concurrent with the higher feed intake and greater body weights, the lipid content and thus energy content of the grouper gradually increased, as well (Table 3).

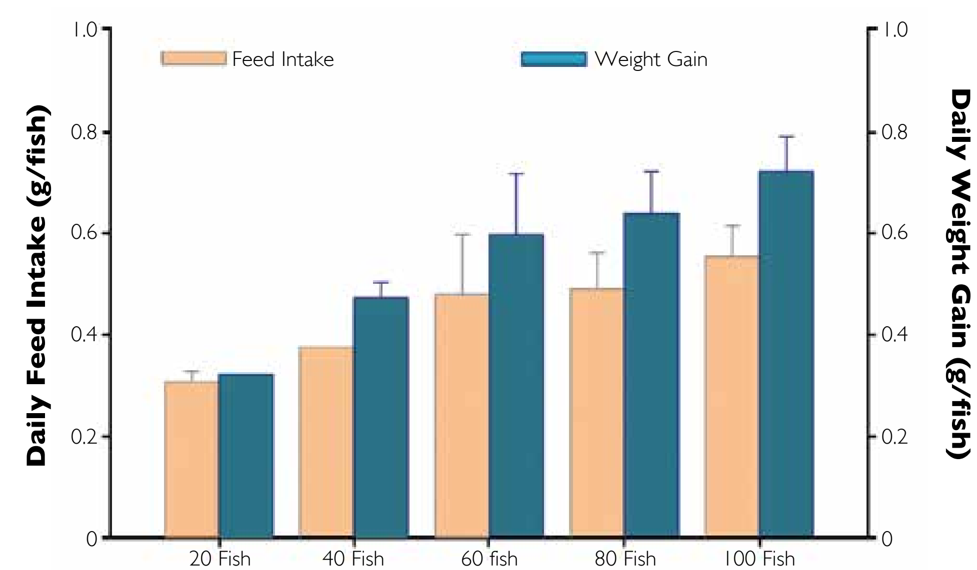

Figure 1. Effects of stocking density on voluntary feed intake and subsequent weight gain in white grouper.

The improved feed intake and subsequent growth performance of the grouper probably reflected a behavioral response to increased crowding in the tanks. At higher fish densities, there might be a disruption of feeding hierarchies among the fish. It is also possible there was some kind of feeding reinforcement among the fish, similar to the feeding frenzies seen in larger groups of fish.

As shown in Figure 1, with increasing stocking densities, feed intake increased, and consequently growth improved. This response was almost linear over the range of stocking densities tested and did not reach an apparent maximum. These results were in contrast to the general perception, where crowding negatively influences growth performance.

Water Quality

Apart from varied responses to increased stocking density among species, the effect of deteriorating water quality associated with higher density might be one of the reasons for suboptimal growth performance for several fish species described in the literature, rather than the stocking density, per se.

Flow-through and cage culture production systems are commonly subject to large fluctuations in water quality. However, in intensive recirculation systems, where water quality parameters are controlled and maintained, it is possible to evaluate the possible effects of stocking density separately from those of other water quality issues.

Another interesting aspect in this study was the improved feed-conversion ratio (FCR) achieved with the increased feed intake. FCR improved with increasing stocking densities from 0.95 down to 0.77 (Table 2). This was contrary to the common belief that fish fed to maximum voluntary intake will display a deteriorating FCR.

The reason for the better FCR was that, relatively speaking, the maintenance requirement of the faster-growing fish represented a smaller proportion of the overall requirement. The opposite is also true – with the higher maintenance demand in relation to weight gain of the slower-growing fish, the FCR will worsen.

Study: Inbreeding Affects Body Weight, But Not Survival In White Shrimp

The study at Maricultura del Pacífico hatchery in Los Pozos, Sinaloa, Mexico, produced three successive generations of families.

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Summary:
Inbreeding commonly causes a decrease in the mean values of traits of productive interest in aquaculture. In a study designed to generate inbred families of Pacific white shrimp, which were the product of sibling mating in successive generations, as well as groups of families with different inbreeding coefficients, there was a significant negative effect on body weight in shrimp at 130 days of age. However, the results showed that inbreeding had no effect on growout survival from 65 to 130 days of age.

Domesticated stocks and breeding programs to improve production and profitability in penaeid shrimp aquaculture are becoming increasingly common. The use of selection programs implies that only a portion of the population contributes genes that are transmitted to the next generation, reducing effective population sizes.

This reduction implies an increased probability of mating between relatives, making a certain degree of inbreeding unavoidable over time. Inbreeding is often associated with a reduction of the mean phenotypic values of some traits, principally those related to fitness and physiological efficiency.

Inbreeding – the result of mating between relatives – can be expressed through an inbreeding coefficient, which ranges between zero and 100%. Inbreeding commonly causes a decrease in the mean values of traits of productive interest.

The magnitude of inbreeding depression varies depending on the trait studied, the population and the level of inbreeding achieved. Therefore, to design efficient breeding programs for shrimp, it is necessary to quantify the effects of inbreeding and incorporate this information in a way that allows proper balancing of the response to selection and the effects of inbreeding.

Inbreeding Study

The authors performed a study with a large number of families to investigate the effects of inbreeding on the body weights of Pacific white shrimp, *Litopenaeus vannamei*, at harvest size after 130 days and on growout survival from 65 to 130 days of age. The experiment was conducted at the Maricultura del Pacífico hatchery in Los Pozos, Sinaloa, Mexico, from 2010 to 2012. It produced three successive generations of families.

The experiment was designed to generate inbred families, which were the product of sibling mating in successive generations, as well as groups of families with different inbreeding coefficients, including families with relatively low inbreeding levels. Mating was carried out to obtain families with an inbreeding level ranging between zero and 61%.

A total of 16,361 shrimp from 320 families were produced. The numbers of animals obtained for each inbreeding interval by year are shown in Figure 1. Inbred families were mainly the product of mating between siblings and cousins (Figure 2), while the rest of the families were the product of several mating schemes between individuals with different levels of genetic relationship.

All mating was performed using artificial insemination. Inbreeding coefficients were calculated using pedigree information, which comprised 11 generations.

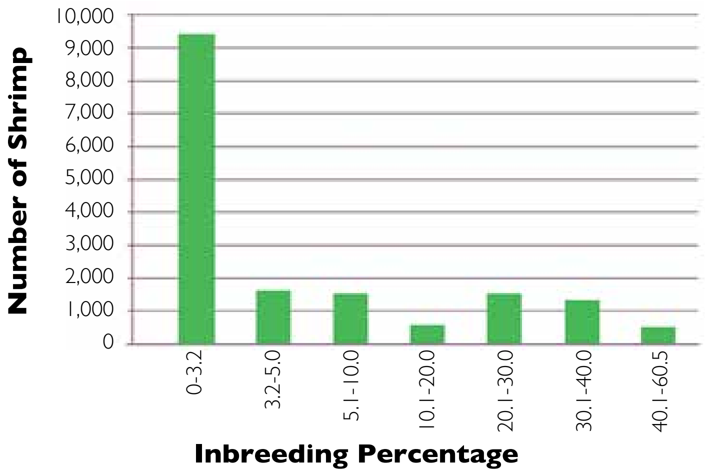

Figure 1. Number of shrimp by inbreeding percentage intervals.

Table 1. Inbreeding effects on body weight and mean survival in Pacific White shrimp per 10% increase in inbreeding coefficient.

Trait	Mean	Inbreeding Effect (%)
Body weight at 130 days of age (g)	19.6	-2.2 (significant)
Survival from 65 to 130 days of age (%)	81.7	-0.01 (not significant)

The effect on each trait per 10% increase in the inbreeding coefficient was estimated. For the analysis, the effects that can influence these traits were taken into account using appropriate statistical methods.

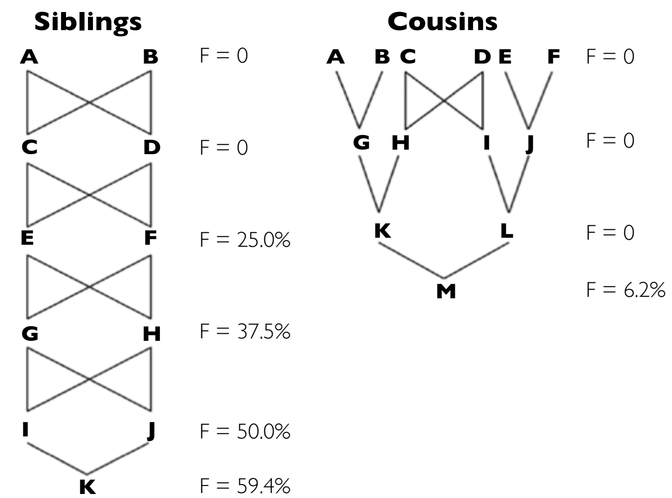

Figure 2. Examples of mating between siblings and cousins, and approximate levels of inbreeding (F) obtained.

Results

There was a significant negative effect of inbreeding on body weight. The estimated change on body weight per 10% increase in the inbreeding coefficient was -2.2% of the mean (Table 1). Survival is a fitness trait, and inbreeding is considered to affect this type of traits. However, these results showed that inbreeding had no effect on growout survival (Table 1).

From these results, a loss of up to 6% of the genetic progress can be expected as an effect of inbreeding in a selected population of Pacific white shrimp. This takes as reference the commercial population of Maricultura del Pacífico, where measures to limit inbreeding are taken.

It should be noted that the inbreeding depression results obtained in this study were very similar to those obtained on the commercial line from the same company. The range of unintended inbreeding coefficients, which result from routine breeding program management, was much smaller – zero to 15%.

These results were obtained in a 2013 study that involved a retrospective analysis of data from eight generations. In that study, the decrease in body weight at 130 days of age per 10% increase on the inbreeding coefficient was -3.4% of the mean, and statistically significant. Similarly, inbreeding had no effect on growout survival rate.

Perspectives

In this population, the inbreeding effect on body weight was relatively small. However, the effect of inbreeding on all important traits must be considered to make the best decisions regarding its control.

To control long-term inbreeding in a closed population, it is necessary to use a relatively large number of broodstock in each generation and control mating among relatives by imposing mating restrictions. These measures may imply some reduction in genetic progress, but allow preservation of the genetic variation necessary to continue improving the population for current traits or others that may need to be incorporated in the future.

A loss of up to 6% of the genetic progress can be expected as an effect of inbreeding in a selected population of Pacific white shrimp.

Natural Feed Additive Improves Shrimp Productivity In Ecuador Demonstration

The inclusion of multi-action feed additives to shrimp diets can improve survival and overall crop yield.

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Summary:
The digestive systems of shrimp are main entry points for bacterial and viral pathogens, and unfavorable microflora. Natural feed additives that combine different actions have proven effective in improving survival where shrimp are exposed to bacterial pathogens. Although not statistically different under the study conditions, a pilot-scale demonstration found that feed supplementation with a gut health modulator resulted in improved values for survival, crop yield and feed conversion. The improved survival was in line with observations made in past trials.

Despite the apparent success story reflected in the past and projected expansion of shrimp aquaculture, production in many regions continues to suffer economic losses due to the impacts of a wide variety of diseases. White spot syndrome (WSS), one of the main causes of the stagnating shrimp industry in the 1990s, continues to affect production in Mexico, Central America and South America, where the more extensive farming methods in large ponds do not allow effective biosecurity measures against the virus that causes the disease. The shrimp production in Southeast Asia and Mexico has been ravaged since late 2012 by a new disease known as early mortality syndrome (EMS or acute hepatopancreatic necrosis disease).

Shrimp, Microflora

Shrimp actively "graze" on the bottom substrates of ponds and are therefore highly exposed to exchanges of microflora between the environment and their digestive systems. This increases the risk for proliferation of unfavorable gut microflora, which can affect digestive functioning.

Furthermore, the digestive systems of shrimp are main entry points for the bacterial and viral infections that remain a major risk for profitable shrimp production. The use of antibiotics to control microbial development throughout the production process is not desirable due to the risk for building up resistance and its rejection by legislators and consumers.

The shrimp industry requires alternative ways to control the microbial ecosystems in production systems. Sustainable approaches to modulate the gut microflora in shrimp include the use of a variety of natural compounds capable of modulating the microflora toward a favorable composition, such as probiotics, organic acids, yeast extracts and phytobiotics.

These strategies may have synergistic effects. For example, phytobiotics can enhance the establishment of probiotic bacteria and therefore enhance the efficacy of probiotic inoculations in the production system.

Functional Feeds

Functional feeds containing gut health promotors deliver with every meal an adequate concentration of natural antimicrobial activities into the shrimp gut. These feeds are a key component of any strategy to prevent diseases, particularly when opportunistic bacteria are a major cause of mortality. However, the success of this approach depends on the efficacy of the selected gut health promoter.

Gut-modulating feed additives ideally are heat stable and can therefore be easily incorporated into feed at the feed mill and be present in every meal from the starter feed onwards, without requiring major adaptions of production protocols at the nursery or farm.

The second author and Dr. Tim Goossens reported in the January/February 2014 issue of the *Global Aquaculture Advocate* that natural feed additives combining different action mechanisms, such as direct bactericide/bacteriostatic properties, as well as quorum sensing inhibition properties at concentrations below minimum inhibitory concentration, are most promising to reduce the impacts from bacterial diseases such as vibriosis.

Farm Trial

The authors performed a pilot-scale farm trial earlier this year to evaluate the effects on shrimp productivity of a feed additive with multiple actions on gut health. The trial was executed at the experimental station of a shrimp farm located in the Guayas Province of Ecuador during the period of February to April. During the trial, two treatments differed only with regard to the addition of a commercial phytobiotic additive to the standard 35%-protein feed used at the farm. The additive was added by top dressing at 3 g/kg of feed using a commercial binder throughout the trial period.

The experimental ponds of approximately 170-m^2 area were seeded with shrimp of an average size of 70 mg at a density of 10/m^2. The test treatment and control were run in five and three replicated ponds, respectively, for 78 days. The feed was applied once daily during the morning following the same fixed table for all ponds. Pond management followed the routine production protocols of the farm.

Although the inherent variation among ponds ($P > 0.05$ for all production variables) did not allow the detection of statistically significant differences, the trial showed some interesting trends (Table 1). The supplementation of the gut health modulator resulted in values that were 20.5% higher for survival, 14.1% greater for crop yield and 14.9% better for feed conversion than the values in the control treatment. However, shrimp that received the feed additive showed 4.7% slower growth.

Table 1. Results at harvest for control ponds and treated ponds after 78 days of culture.

Treatment	Survival (%)	Shrimp Size (g)	Crop Yield (kg/ha)	Feed-Conversion Ratio	Weekly Growth (g)
Phytobiotic	57.4 ± 10.8	15.6 ± 1.0	885 ± 149	1.20 ± 0.20	1.40 ± 0.09
Control	47.3 ± 3.1	16.4 ± 0.7	776 ± 83	1.41 ± 0.14	1.47 ± 0.06
Change	20.5%	– 4.7%	14.1%	–14.9%	– 4.7%

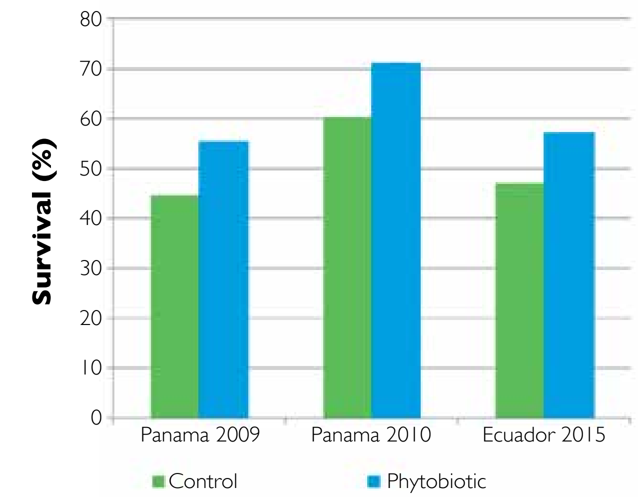


Figure 1. Comparison of the effects of a functional feed additive with combined antibacterial/quorum sensing inhibition action on survival of *Litopenaeus vannamei* in field trials under semi-intensive conditions.

Other Studies

In these production trials, the main disease challenges at the farm consisted of WSS and vibriosis. The improved survival relative to the control was in line with observations made in past trials in Panama (Figure 1).

Natural feed additives that combine different action mechanisms against *Vibrio* species have proven effective in improving survival under challenging situations where shrimp are exposed to bacterial pathogens. In a 2011 paper presented at the International Shrimp Culture Symposium and Exhibition, Dr. Jorge Cuellar said that compared to results for controls, the inclusion in a standard pelletized feed of the botanical feed additive used in the current study improved survival 24% and 18% during two independent production cycles at a semi-intensive shrimp farm in Panama.

The negative effect on growth observed in the current trials needs further study. In semi-intensive farming, the use of fixed feeding tables often causes an interference between survival and growth due to the lack of correction of feed availability per shrimp in function of survival. This often results in a growth limitation in the treatment showing the best survival.

In a study by Dr. Loc Tran and collaborators reported in the May/June *Aqua Culture Asia Pacific*, the researchers confirmed the effects of the same synergistic phytobiotic product in a controlled challenge trial with a pathogenic EMS strain of *Vibrio parahaemolyticus* under laboratory conditions.

The researchers reported 62 to 107% increased survival in shrimp that received the additive during three weeks prior to the experimental infection, compared to unsupplemented control groups. The addition of the phytobiotic product in the diet resulted in consistently lower *Vibrio* counts in the digestive systems of the shrimp that consumed the diets compared to the counts in the control shrimp, illustrating the capability of gut-modulating additives to protect shrimp's gut flora throughout a *Vibrio* challenge.

In semi-intensive farms, the use of fixed feeding tables often causes an interference between survival and growth due to the lack of correction of feed availability.

Aquafeed inputs drive aquaculture production systems. Uneaten or undigested feed and its metabolic by-products are the primary contributors to water quality issues. Proper feed and feed management techniques require continuous monitoring, review and improvement.

Feeds And Water Quality Revisited

Summary:
Uneaten or undigested feed and their metabolic by-products are the primary contributors to declines of water quality in aquaculture systems. Accordingly, feed and feeding techniques require continuous review. Reducing overfeeding is an important opportunity for improving feed conversion in most production systems. Managers should apply highly digestible, nutrient-dense feed formulated to avoid nutrient excesses and nutrient deficiencies. Maintain optimum physiology and health of animals' digestive tracts to be sure nutrients move from feed into the lymphatic or circulatory systems through digestion and absorption.

Although there are volumes of articles and printed material on the importance of maintaining optimum water quality in aquaculture farming systems, including related remedial technologies, there are still too many industry reports of crop failures or low production resulting from undesirable water environments.

Aquaculture production conditions have changed over the last 40 years. Animals are growing faster, stocking densities have increased and containment types have changed, as have management methods – all resulting in greater biomass harvested per unit of time and space. These changes are necessary in order to maintain an economically viable industry.

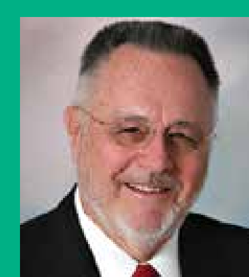
To accommodate many of these changes, feed rates have increased – resulting in higher oxygen demand; greater carbon dioxide, ammonia and nitrite production; and materially increased levels of dissolved, suspended and settleable solids. These water quality factors rapidly reach concentrations that are not tolerable for the culture animals. Therefore, farm managers must adapt new and improved methods and procedures. These can be preventive methods or remedial methods.

Feed is the primary cost factor for aquaculture production.

Feed drives the system. Uneaten or undigested feed and its metabolic byproducts are the primary contributors to the rapid decline of water quality. Accordingly, feed and feeding techniques require continuous review and improvement.

Feed Efficiency

Efficiency is technically defined as output divided by input. In aquaculture, it would be defined by units of gain divided by units of feed input, or feed-conversion efficiency (FCE). This metric is primarily used in scientific publications, but for reporting in the industry, the commonly used metric is the feed-conversion ratio (FCR), which defines the units of feed required to produce a unit of weight gain.



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It is important to understand the difference between FCE and FCR. A feed with a higher FCR results in less of the feed going to tissue growth and more of the feed going into the water environment. Conversely, a feed with a lower FCR would have more of the feed going into tissue growth and less into the water column.

FCRs must be evaluated in real time, such as on a daily or weekly basis, in order to be relevant. If one just uses the FCR values at the end of a crop, this number has economic importance, but its value in terms of measuring feed efficiency is compromised because of the impact of mortality unless the weight of the dead animals is added to the weight of the crop.

Due to the application of best nutritional and feed-manufacturing practices, today's feeds are capable of resulting in a real-time FCR of 1.1:1 in most commercially produced shrimp and fish. This should be the goal in managing farms and culture practices.

Old Is Good

Thirty-seven years ago, Dr. George Klontz and associates published a research report entitled "Aquaculture Techniques: Water Use and Discharge Quality." The objectives of this report were to develop and test methods of predicting waste product generation from aquaculture facilities. From their studies, they were able to develop a method to estimate the amount of solids produced from a known quantity of feed, shown in Table 1.

This table dramatically illustrates that as FCR increases, the amounts of solids going into the water column also increase significantly. An increase in FCR of 0.2 units increases the amount of solids by approximately 8.5 kg/100 kg feed fed. An increase in FCR from 1.2 to 1.6 increases the amount of water solids by 19.7 kg/100 kg feed.

The quantity of waste predicted is reported as solid waste and does not consider soluble wastes that dissolve from the waste solids. All of this extra waste must be dealt with in some manner, at a cost, in order to maintain water quality. If the waste collects at the bottoms of ponds or containments, it can produce hydrogen sulfide, which is highly toxic to aquatic animals and puts the entire crop at risk.

Although Klontz conducted his work with trout, it is suggested that as FCRs increase, the relative increases in the amounts of solids produced would be similar for most similar aquaculture species, including shrimp.

Managing FCR

Reducing FCRs without compromising animal performance, growth rate and survival should be a continued objective of management. Several important tools and techniques can be applied to accomplish this objective.

- Reducing or eliminating overfeeding is perhaps the single most important opportunity for reducing FCRs in most aquaculture production systems. This involves selecting the right highly palatable, complete feed; selecting the right particle size; and after considering the environment and physiology and behavior of the species, feeding the animals the way they would like to be fed. Usually, more frequently is better – and be sure that animals can easily and quickly access the feed when delivered.
- Feed a nutrient-dense feed formulated to avoid both nutrient excesses and nutrient deficiencies. Antinutritional factors must also be absent.
- The feed should be highly digestible and formulated using highly digestible ingredients. The scientific literature reports that the digestibility of dry matter and protein can vary 20% or more for different sources of the same ingredient. Undigested material that passes through animals without contributing any benefit results in increased FCRs and the amounts of solids introduced to the water environment.
- Maintain optimum physiology and health of animals' digestive tracts, for there is where nutrients move from feed into the lymphatic or circulatory systems through the processes of digestion and absorption. If the lining of the digestive tract becomes irritated and dysfunctional as a result of toxins or bacterial or parasitic infections, the digestive processes are reduced. The digestibility of feed becomes impaired, resulting in greater fecal waste.

Reducing or eliminating overfeeding is perhaps the single most important opportunity for reducing FCRs in most aquaculture production systems.

Although it is well known that underfeeding normally reduces FCRs, this practice is definitely not recommended for several reasons. Hungry animals will consume the detritus on pond bottoms, which can be a significant source of disease. Hungry animals will also aggressively consume small, weak or dead animals, which can also be infected with disease organisms. If underfeeding is not carefully controlled, it will result in smaller, less-uniform animals and lower biomass at harvest. The resulting economic loss can easily be greater than the value of the feed saved.

Perspectives

A clear understanding of the roles that feed and feeding have on water quality is important knowledge for the producers of aquatic animals. Feed-conversion ratios are a useful metric to measure whether effective production techniques are in place.

A numerically lower FCR is better, as it normally indicates higher levels of feed utilization and lower levels of solids going into the water column. It can also indicate that less feed is being wasted.

Preventing water quality problems through proper feeds and feeding should be a top priority, as the cost of remediation can be quite significant. In all cases, water quality standards for the optimum performance of aquatic animals must be maintained. Mother Nature does not make compromises.

Bottom Line: Prudently reduce FCRs and improve water quality!

Table 1. Estimated solids generated per 100 kg feed fed. From G. W. Klontz, I. R. Brock and J. R. McNair (1978).

Feed-Conversion Ratio	Dietary Efficiency (%)	Solids (kg)
1.1	90.9	8.7
1.2	83.3	15.9
1.3	76.9	22.0
1.4	71.4	27.2
1.5	66.7	31.4
1.6	62.5	35.6
1.7	58.8	39.1
1.8	55.6	42.2
1.9	52.6	45.0
2.0	50.0	47.5
2.1	47.6	49.8
2.2	45.5	51.8
2.3	43.5	53.7
2.4	41.7	55.4
2.5	40.0	57.0

Efficiency Of Mechanical Aeration

Although widely implemented, the long-arm paddlewheel aerators typically used in Asia do not reflect the most efficient designs.

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Summary:
Although relatively expensive, mechanical aeration increases the amount of aquaculture production possible per unit area. Aerators are rated according to standard aeration efficiency (SAE), an expression of how much oxygen is transferred. Since SAE test conditions are seldom met in culture, actual oxygen-transfer rates are 40 to 60% of the SAE values. Asian-style paddlewheel aerators are widely used but reflect design and operational issues that limit efficiency. Testing has led to more efficient designs that are now widely used in U.S. catfish and shrimp ponds.

Mechanical aerators are used increasingly in aquaculture because aeration can greatly increase the amount of production possible per unit area or volume of water. These devices usually are powered by electricity, but in some locations, small diesel engines are the power source.

During a recent visit to a shrimp-farming area in Thailand, the author saw ponds aerated at 24-36 hp/ha (18-27 kW/ha). These aerators often are operated about 20 hours daily over a 60- to 100-day crop period. At a farm with 24 hp/ha of aeration and a 100-day crop, about 36,000 kWhr of electricity would be used for aeration.

Shrimp production for successful crops of 14- to 18-g shrimp was reported to be around 7,000-9,000 kg/ha. Electricity costs about U.S. $0.10/kWhr in Thailand. Thus, aeration costs $0.41 to $0.53/kg of shrimp for electricity alone. Aeration costs for fish production usually are lower than for shrimp, but still represent a major production expense.

Oxygenation Efficiency

Because of the high cost of mechanical aeration, why has there not been more effort to improve the oxygen-transfer efficiencies of mechanical aerators and determine how to use these devices more efficiently in ponds? There is a relatively simple procedure for testing the oxygen-transfer efficiency of aerators.

Water in a large tank is deoxygenated with sodium sulfite and a small amount of cobalt chloride to catalyze the reaction of sulfide with oxygen. The aerator under test is then operated to reoxygenate the water. The dissolved-oxygen concentration in the tank is measured at frequent intervals while it increases from 0 mg/L to 70 or 80% of saturation. A mathematical procedure is used to estimate the oxygen-transfer coefficient, the slope of the reaeration line.

Standard Aeration Efficiency

The tank volume, water temperature, oxygen-transfer coefficient and power input to the aerator during the part of the test used in estimating the oxygen-transfer coefficient allow calculation of the standard aeration efficiency (SAE) of the aerator. SAE is an expression of the amount of oxygen that an aerator will transfer at 20° C to clean freshwater containing 0 mg/L of dissolved oxygen. SAE can be reported in any of the following units: lb oxygen (O_2)/hp/hour, lb O_2/kW/hour, kg O_2/hp/hour or kg O_2/kW/hour. The SAEs for aerators range about 0.5-2.0 O_2/kW/hour.

SAE is analogous to gas mileage ratings for cars. These ratings are determined by a highly standardized procedure in the laboratory, but passenger cars are operated under much different and varying conditions. One would not expect a car to actually use fuel at the same rate as it did in the standard test. Nevertheless, a car with a better fuel use rating will use less fuel than a car with a poorer fuel use rating when both are driven under similar road conditions by the same driver.

In an aquaculture system, the conditions existing in the SAE test will not occur. The water will not be clean, the temperature likely will not be 20° C, and dissolved oxygen certainly will be present. Nevertheless, the SAE is particularly valuable for comparing the performance of mechanical aerators. Under identical conditions, an aerator with a higher SAE will transfer more oxygen to the water than will an aerator with a lower SAE.

An equation is available for estimating the oxygen-transfer rate of aerators under actual culture conditions if the SAE of the aerator is known. In most culture systems, the actual oxygen-transfer rate is 40 to 60% of the SAE (Table 1).

Table 1. Factors for estimating actual aeration efficiency (AAE) from standard aeration efficiency (SAE) in freshwater at 28° C. AAE = SAE x factor.

Dissolved-Oxygen Concentration (mg/L)	Factor
0	0.95
1.0	0.87
2.0	0.74
3.0	0.62
4.0	0.49
5.0	0.36
6.0	0.23
7.0	0.10
7.8	0

Calculating SAE

In the late 1980s, an effort was initiated at Auburn University to develop an efficient aerator for channel catfish ponds. Several types of commercially available aerators were tested, and the paddlewheel aerator had the most potential for improvement.

A device was fabricated that allowed the testing of an array of paddle shapes, paddlewheel diameters, numbers of paddles per row around the aerator hub, paddlewheel speeds, paddle depths, paddle positioning on the hub and the amount of power input necessary for each combination. The resulting data were used to calculate SAE and determine the efficient paddlewheel design that is illustrated in Figure 1.

The optimum operating conditions established in the tests varied with aerator size, but the 10-hp paddlewheel illustrated in Figure 1 has become the standard aerator for catfish farming. This paddlewheel usually is operated at 80 to 90 rpm with paddle depth submergence around 8 to 12 cm.

The SAE of the paddlewheel aerator is typically around 2 kg O_2/kW/hour, and even higher values have been reported. Smaller paddlewheels fabricated according to this basic design also have similar SAEs.

Cost, Design Issues

Paddlewheel aerators of the design shown in Figure 1 are used widely in the United States in catfish and shrimp ponds. However, apparently because of the greater initial cost, this type of aerator is not used as commonly as Asian paddlewheel aerators in other countries.

Tests showed that these typical paddlewheels have lower SAEs – usually no more than 1 kg O_2/kW/hour. The main reasons for the lower SAEs are related to the design of the paddles and paddlewheels.

The paddles have many holes in them. These holes lessen the amount of water splashed into the air to affect oxygenation, and water passing through the holes increases friction loss – also making oxygen transfer less efficient. There are typically six or eight paddles per row on the aerators. Tests have revealed that four paddles per row were more efficient.

Another problem with many paddlewheel aerators used in shrimp ponds in Asia is that the power of the electric motor or diesel engine used to power the devices often is not well matched

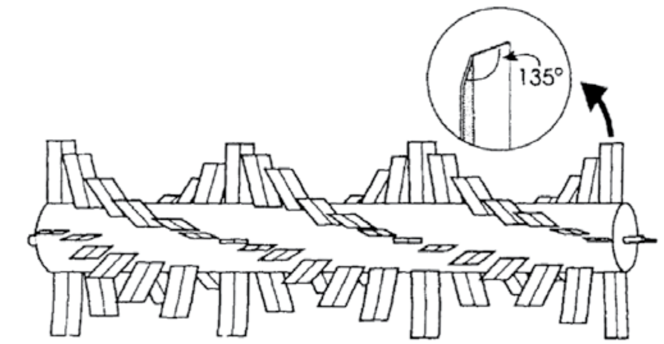

Figure 1. This highly efficient paddlewheel design, which resulted from extensive testing at Auburn University, is widely used in pond aquaculture in the United States. It features multiple rows of staggered paddles.

with the load imposed by the rotating paddlewheel. The mismatch of power units and paddlewheels is particularly evident for the long-arm aerators used in Thailand.

Because aeration is a major expense in aquaculture, there is incentive to improve aeration efficiency and lessen costs. It also is important to note that it is usually more costly to use diesel-powered aerators than electric ones. To provide 1 kWhr of power to the shaft of an aerator using a small diesel engine with a typical mechanical efficiency of 0.3 would require an energy input of about 12 megajoules (MJ)/hour. About 0.335 L of diesel fuel per hour would be needed. At a fuel cost of U.S. $0.97/L, this much fuel would cost about $0.32. One kilowatt hour of electricity typically costs $0.10 to $0.15.

Perspectives

Much improvement in aeration efficiency could be achieved by modifying the designs of Asian paddlewheel aerators through adoption of paddle and paddlewheel design features already shown to be efficient through testing. Where possible, diesel-powered aerators should be phased out in favor of electrically powered ones.

Aeration often is not applied at high enough rates to prevent dissolved-oxygen concentrations from falling low enough to stress the culture species at night. Conversely, excessive aeration may be used in the daytime, when dissolved-oxygen concentrations usually are adequate. Thus, greater effort should be devoted to developing better operational strategies for aerators.

In addition to lessening aeration costs per unit of aquaculture production, more efficient aeration would lessen energy input, embodied resource use and negative environmental impacts associated with energy use for aeration. Moreover, those involved in aquaculture ecolabel certification should consider including the efficiency of aeration in the standards for these programs.

Editor's Note: The author has offered to provide the procedure for measuring the SAE of mechanical aerators to anyone interested in conducting aerator performance tests.

In addition to lessening aeration costs per unit of aquaculture production, more efficient aeration would lessen energy input, embodied resource use and negative environmental impacts.

Biofilter Inoculation In Recirculating Aquaculture Systems

The experimental system used multiple fish tanks with effective water treatment and system control devices.

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Summary:
Biological filters are essential parts of recirculating aquaculture systems that transform toxic fish compounds such as ammonium and nitrite into less-harmful nitrate. The authors tested the convenience and efficiency of three methods for the initial inoculation of aerobic biofilters. Only the system stocked with fish at low density showed stable nitrification and low ammonia and nitrite concentrations. A system equipped with used filter media started nitrification immediately, while ammonia and nitrite concentrations in a system with a commercial solution containing nitrifying bacteria remained high.

A properly operating recirculating aquaculture system (RAS) consists of various modules, all essential for the culture of aquatic organisms under appropriate conditions. These systems combine mechanical, biological and chemical treatment to ensure good water quality and enable the reuse of the water.

The excretions of fish, which include dissolved ammonia as well as particulate organic matter, can lead to the accumulation of toxic compounds that endanger the well-being of the culture animals. Applying mechanical filtration via drum or sand filters can remove most of the particulate matter, while biological filtration converts dissolved toxic compounds into less-harmful matter through the metabolism of nitrifying bacteria kept within the biofilter. Other cleaning devices include protein skimmers, ultraviolet irradiation and anerobic biofilters for denitrification.

Experimental Setup

Depending on the applied conditions within a system, the start of a stable bacterial community is time consuming and can take between weeks and months. The authors performed a study to test the convenience and efficiency of methods for the inoculation of an aerobic biofilter when new biofilters are brought into operation. Three different methods were applied to three identical new recirculating aquaculture systems.

Each RAS consisted of three, 1.1-m^3 fish tanks, a cartridge filter, an aerobic moving-bed biofilter for nitrification, an anaerobic moving-bed biofilter for denitrification, a protein skimmer with the addition of ozone, a sump and several water and air pumps. Each system had a total water volume of 5.3 m^3 and was connected to a central cooling system via a plate heat exchanger to keep water temperature constant.

The individual biofilters had water volumes of 0.75 m^3, filter media volumes of 0.54 m^3 and active surface areas of about 315 m^2.

Filter Inoculation

In the first system (S1), 10% of the new filter media was replaced by media from another RAS that was already colonized with nitrifying bacteria. System 2 (S2) was stocked with turbot, *Scophthalmus maximus*, at a low density. The last system (S3) was inoculated with a commercial bacteria solution according to the producer's recommendations. Each system was supplied with the same amount of nutrients to feed the aerobic biofilters for a period of 13 weeks. S1 and S3 were supplied with dissolved ammonium chloride based on the fish excretions in S2.

Results

The abiotic conditions during the experiment were stable and in the recommended range for the culture of coldwater fish such

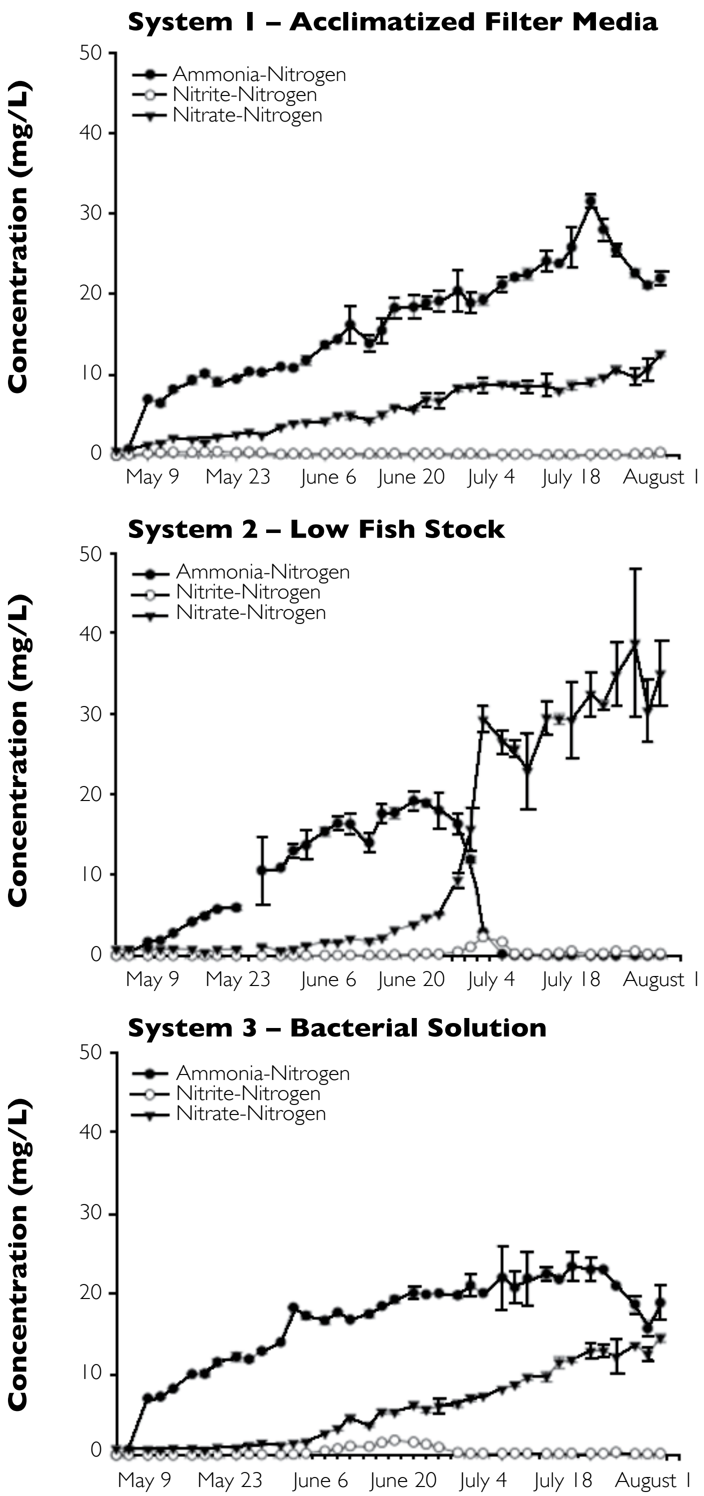

Figure 1. Concentrations of dissolved nutrients during the course of the experiment.

as turbot. Temperatures ranged between 14.5 and 16.0° C, pH levels decreased slightly from 8.1 to 7.5, and oxygen saturation kept stable just above 100%.

Figure 1 shows the nutrient concentrations for the individual systems. S1 showed high ammonia-nitrogen (ammonia-N) concentrations of about 20 mg/L and rising nitrate-nitrogen (nitrate-N) concentrations near 12 mg/L at the end of the experiment.

Stocked with fish at a low density, S2 showed very low ammonia-N concentrations near 0.05 mg/L and high nitrate-N concentrations of about 35 mg/L. S3, inoculated with a bacterial solution, also showed high ammonia-N levels of about 19 mg/L and intermediate nitrate-N concentrations near 15 mg/L. The nitrite-N concentrations for all systems peaked before the end of the experiment and leveled in the range of 0.2-0.4 mg/L.

Discussion

The temperature applied for the start of the biofilters was recommended for the culture of turbot. However, for the start of an aerobic biofilter at such low temperatures, more time is required to reach stable nitrification processes.

S1 achieved nitrification almost immediately after inoculation due to the nitrifying bacteria previously established on the filter media. This caused an early and relatively low nitrite peak three weeks after inoculation. Yet during the course of the experiment, ammonia-N concentrations consistently increased, peaked after week 11 and decreased thereafter.

S2 revealed the classic data curves for a starting nitrifying biofilter, with an ammonia peak a few weeks after the start of the system, followed by a short nitrite peak and finally an increasing nitrate concentration. The inoculation of S2 was completed after 10 weeks.

S3 showed a slow increase in nitrite-N and nitrate-N concentrations, which were considerably lower than those of the other systems. The nitrifying bacteria within the solution likely would have had to settle before their metabolism would be able to efficiently convert the ammonia-N into nitrite-N and nitrate-N.

Several factors probably increased the time to establish acceptable and stable nitrification processes. All the biofilters were moving-bed designs. The constant movement of the filter media caused abrasion, which scraped off bacteria mats and therefore prolonged successful bacterial colonization. Furthermore, the protein skimmers led to a reduction of free bacteria that otherwise would settle within the system. This was caused by the foam formation and subsequent removal from the system.

Perspectives

Starting a new filtration system can require considerable time and should be arranged well in advance. The authors recommend that new biofilters should be inoculated by stocking the intended culture species at low density for several weeks, depending especially on the water temperature. Reducing the air supply to the biofilter will reduce the abrasion and therefore increase the settling rate of nitrifying bacteria. A shutdown of the protein skimmer during inoculation of the biofilters could also help keep the bacterial count high, but a total shutdown can cause other problems, such as coloration of the water. Therefore, an ozone reduction could also help.

Combining the different methods can shorten the required time to reach stable conditions. Adding used filter media to a system stocked with a low biomass can lead to the immediate start of nitrification. Adding a bacterial solution to a system with limited fish stock can introduce more desired nitrifying bacteria to the system and therefore speed up the operation time of the system.

Starting a new filtration system can require considerable time and should be arranged well in advance. The authors recommend that new biofilters should be inoculated by stocking the culture species at low density for several weeks.

Dietary Acidification In Aquaculture

Tilapia after a trial.

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Summary:
Much of the chemical breakdown of foodstuffs begins in the stomachs of animals through acidification. Stomach pH remains elevated after feeding, with the buffering capacity of the feed a major contributor to the increased pH. Fishmeal-based diets need more energy for acid secretion than diets without animal proteins. Continuously fed fish had minimum stomach pH levels that support the activation of pepsin, which digests protein. Effective use of digestive biology is a goal in aquaculture, so dietary acidifiers have been gaining interest in recent years.

Digestion is the mechanical and chemical breakdown of food into its metabolizable parts, which can be used by an organism for energy, growth and/or reproduction. In monogastric animals, which include a wide variety of aquaculture species (for example, salmon, trout, tilapia, seabass and *Pangasius*), much of the chemical breakdown of foodstuffs begins in the stomach through acidification.

Digestive Process

In most monogastric animals, once food enters the mouth, it is moistened by saliva, and the digestive process begins. After being swallowed, the food passes down the esophagus into the stomach, where stomach acid and enzymes begin to break it down. The mixture of food and the various intestinal secretions is known as "chyme." Bile salts stored in the gall bladder and enzymes and alkali secreted by the pancreas mix with the emptied stomach contents in the small intestine, where the stomach acid is neutralized, and most fats are broken down.

The "stomach acid" working in all monogastric animals is hydrochloric acid, a very strong inorganic acid produced by gastric glands (parietal cells). This acid is able to lower the pH in the stomach to levels between 2 and 3, depending on species and diet.

Hydrochloric acid production at birth/hatching is negligible, but increases during the growth of animals. As more acid is produced in the stomach, the pH decreases. Low pH is required for the activation of pepsin, a proteolytic enzyme needed to digest protein. The optimal pH for pepsin activity is 2. At higher levels, its activity is severely reduced.

Implications For Aquaculture

What implications does this have for monogastric aquaculture species, which are heavily dependent on high-protein inputs and the proper digestion of these expensive ingredients – especially keeping in mind that diets containing high amounts of proteins tend to buffer the effects of the hydrochloric acid produced by the body?

In 2009, Canadian researchers Carol Bucking and Chris M. Wood looked into the effects of feeding on stomach pH. The authors fed rainbow trout with mean weights of 350 g a commercial feed with 41% crude protein at 2% body weight ration in a single meal and monitored the resulting pH values in the stomachs of the fish.

Just before feeding, the stomach pH was about 2.7, whereas one hour after feeding, it went significantly up to 4.9. It remained at this level – far above the optimum for pepsin activity – for at least eight hours. The chyme was released into the duodenum eight hours after feeding with pH levels that were far too high.

Buffering Capacity

The authors speculated that the buffering capacity of the feed was a major contributor to the increased pH of the gastric fluids. It took the fish more than 24 hours to reach the "low" initial pH of the stomach.

Lorenzo Marquez and fellow researchers also showed the effects of diet buffering on the gastric acidification in juvenile fish in 2011. They found that fishmeal-based diets had buffering capacities 10 times higher than those in diets without fishmeal. The fishmeal diets therefore needed more energy for acid secretion per digestion cycle than test diets without animal protein meals.

Similar observations on feeding and gastric pH were also made by Manuel Yufera and his team in 2011. This Spanish research group fed juvenile gilthead sea bream in three different ways – once, twice or continuously – and monitored the resulting pH levels in the stomachs of the fish.

Feeding was done either at 9 a.m. and 5 p.m. or continuously between 9 a.m. and 9 p.m. The gastric pH values exhibited significant daily rhythms under the different feeding regimes (Figure 1). Only the regime that offered feed continuously allowed the stomach pH to remain in the region of optimal pepsin for a while. This may also be a reason for the significantly greater weight of the fish in that feeding regime. It could be also explained by the higher

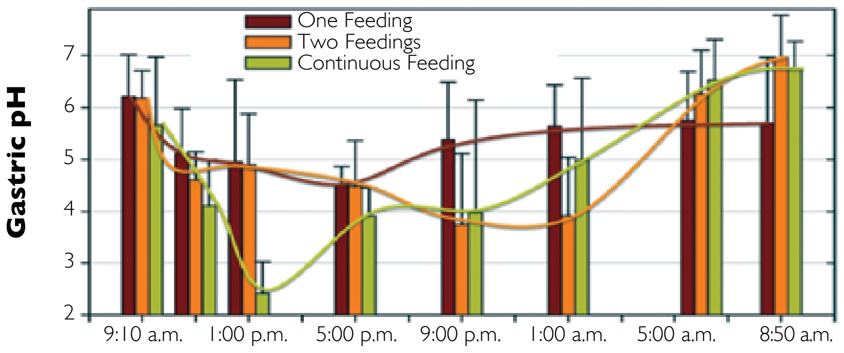

Figure 1. Stomach pH in seabream after feeding using three different feeding regimes. (Adapted from Yufera et al., 2011.)

gastric activity in fish with gastric pH levels below 4.5, as documented by Marquez and colleagues in 2011.

A subsequent study from Yufera's group in 2012 took this a step further. This time, the authors looked at the connection between stomach pH and pepsin activity in juvenile marine fish. Fish were again fed a single meal, twice or continuously with the same diet at the same times as mentioned above.

Fish fed only once again had stomach pH levels around 4.5, while the highest pepsin activity was actually reported before feeding, with 30 pepsin units/fish. In contrast, continuously fed fish reached a minimum pH in the stomach of about 2.5 and had a resulting pepsin activity of almost 280 units/fish in the late afternoon – clearly demonstrating the impact of low pH on pepsin activation in fish.

Aquaculture Applications

Effective use of the digestive biology of fish is of course a goal in aquaculture, so the use of dietary acidifiers has been gaining interest in recent years. However, most of the described effects are thought to stem from the antibacterial action of organic acids, and the impact on protein digestion is often overlooked.

A recent meta-analysis for potassium diformate (KDF) found significantly improved weight gain and feed efficiency for tilapia at levels that can be described as "growth promotion" (Table 1). The final data for that analysis contained the results of eight published studies covering 3,040 fish and comprising 18 trials with KDF inclusion rates from 0.20 to 0.75%.

The average level of dietary potassium diformate from the data set in all treated fish was 0.4%. Only a numerical increase of feed intake (2.1%) could be monitored compared to fish without the additive. However, based on final weight, the performance of tilapia was significantly increased by 5.6%. Furthermore, the feed-conversion ratios of fish fed KDF were also significantly improved by 4.5%.

If calculated using the fish productivity index, the overall improvement amounted to 17.0%. These results may not have stemmed only from the well-documented antibacterial effects. Since acidifiers, if chosen properly, have impacts on buffer capacity and/or stomach pH, they also impact the digestion processes in the gastric tract. This was recently confirmed by a study by Egyptian researchers, who found improvements in the protein efficiency ratio and apparent protein digestibility of 10.3 and 6.8%, respectively, in tilapia fed with dietary KDF.

Table 1. Meta-analysis of the performance of dietary potassium diformate in tilapia versus negative control.

Dosage	Feed Intake	Weight Gain (g)	Feed-Conversion Ratio	Fish Productivity Index*
0.41%	+2.1	+5.6	-4.5	+17.0
Significance	0.16	0.009	0.012	0.02

* Fish productivity index = weight gain (g) x survival (%)/(10 x feed-conversion ratio).

Maximizing Nutrition For Adult Marine Fish

Adult Florida pompano were weighed and measured during harvest.

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Summary:
Since fish consume more feed in the final stages of growout, the development of ecologically efficient and nutritionally adequate diets for adult fish should be a priority. Studies with cobia showed that up to 80% of fishmeal in diets could be replaced with alternative proteins without compromising growth performance or health. Digestible protein:digestible energy ratios are important for optimizing feeding efficiency in feed formulation and aquaculture production. Larger pompano were found to perform well on levels of methionine lower than previously recommended.

Feeds can represent more than 70% of the production costs in aquaculture. Since increased consumption of feed occurs in the final stages of the growout cycle, it is clear the development of ecologically efficient, economically viable and nutritionally adequate diets for adult marine fish should be a priority.

Yet we are still faced with insufficient information on the nutrition requirements of large sizes of commercially important tropical marine fish species and no clear information on the maximum levels of replacement of fishmeal and fish oil in diets for near-market-size fish. Feed manufacturers have made important progress towards formulating and developing better diets, but additional information is required to improve the sustainability and economic viability of the industry.

The aquaculture nutrition program at the University of Miami in Florida, USA, was established in 2010 to provide scientific and technological support in the area of nutrition to companies dedicated to the aquaculture of tropical marine fish. With the support of the United Soybean Board, Illinois Soybean Association, U.S. Soybean Export Council and Open Blue Sea Farms in Panama, the program has conducted research on nutritional requirements and traits of feed ingredients for cobia, pompano and, with Martec S.A. in Costa Rica, spotted rose snappers.

Fishmeal Replacement For Adult Fish

A study on the effects of replacing dietary fishmeal with soy-based products on the production performance of cobia between 1.8 and 3.2 kg was conducted by Suarez et al. (2013. *Aquaculture* 416-417. 328-333). The soy-based products used were dehulled soybean meal, solvent-extracted soybean meal and a novel non-genetically modified variety of soybean meal.

Results indicated that up to 80% of the fishmeal could be replaced in larger cobia, attaining a fish in:fish out ratio of 1.3 without compromising growth performance or health (Table 1). The ability to replace such a high amount of fishmeal indicated the nutritional requirements of cobia may change with age and that developing more cost-effective and environmentally sustainable diets is possible without compromising health or growth rates.

This study helped validate the need for additional research on cobia and others species, not just at the juvenile stage, but at every stage of the commercial production chain.

Digestible Protein:Digestible Energy Ratios

In a study in review with adult Florida pompano by Matthew Taylor, the effects of dietary digestible protein:digestible energy (D.P.:D.E.) ratios on growth, feed efficiency, nitrogen utiliza-

Table 1. Performance indicators for commercial-size cobia fed experimental diets at different sampling periods.

Dietary Treatment	Final Weight (kg)	Weight Gain (%)	Specific Growth Rate (%/day)	Feed Efficiency	Feed-Conversion Ratio	Fish In: Fish Out Ratio
Days 1-29[1]						
1	2.38	35.5[a]	1.02[a]	0.56	1.80	2.08[c]
2	2.58	24.4[b]	0.74[b]	0.51	1.95	1.17[b]
3	2.50	35.6[a]	1.02[a]	0.57	1.81	1.10[b]
4	2.38	33.1[a]	0.97[a]	0.57	1.74	0.91[a]
Pr > F (spell out)	0.24	0.014	0.008	0.82	0.87	0.003
Pooled standard error	0.46	15.04	0.37	0.08	0.28	0.52
Days 2-60[2]						
1	2.80	56.2	0.76[a]	0.49	2.0	2.30[c]
2	3.00	49.9	0.61[b]	0.47	2.1	1.27[b]
3	2.84	52.3	0.69[a]	0.46	2.1	1.31[b]
4	2.81	54.5	0.71[a]	0.49	2.0	1.00[a]
Pr > F (spell out)	0.43	0.1	0.04	0.60	0.69	0
Pooled standard error	0.60	17.8	0.21	0.03	0.13	0.54
Days 1-90[3]						
1	3.15	78.5	0.62	0.41	2.4	2.8[b]
2	3.30	64.1	0.53	0.40	2.5	1.5[a]
3	3.27	75.3	0.59	0.43	2.3	1.4[a]
4	3.10	73.1	0.58	0.39	2.5	1.3[a]
Pr > F (spell out)	0.60	0.24	0.25	0.30	0.31	0
Pooled standard error	0.75	28.9	0.18	0.02	0.13	0.63

Source: Suarez, J. A.; Tudela, C.; Davis, D.; Daugherty, Z.; Taynor, M.; Glass, L.; Hoenig, R.; Buentello, A.; Benetti, D. D., 2013. Replacement of fishmeal by a novel non-G.M. variety of soybean meal in cobia, ***Rachycentron canadum***: Ingredient nutrient digestibility and growth performance. ***Aquaculture*** 416-417. 328-333.
Values in a column with different superscript letters are significantly different ($P < 0.05$).
[1] Initial weight: 1.86 ± 0.3 kg, final biomass density: 6.5 ± 0.3 kg fish/m^3
[2] Final biomass density: 7.6 ± 0.4 kg fish/m^3
[3] Final biomass density: 8.7 ± 0.5 kg fish/m^3

tion and body composition were investigated. A growth trial and subsequent digestibility trial were carried out utilizing five diets based on anchovy meal, soybean meal or corn gluten meal as protein sources. The feeds were formulated to have different D.P.:D.E. ratios ranging 21.7-26.3 mg protein/kJ.

Fish were fed to apparent satiation by hand once daily at 9 a.m. for 88 days. No significant difference between growth performance and liver weight was observed for all diet treatments, except for the diet with 21.7 mg protein/kJ D.P.:D.E., which had significantly lower mean weight gain, feed efficiency and feed conversion.

The digestible protein:digestible energy ratio has become a major factor in fish feed formulation. The Committee on the Nutrient Requirements of Fish and Shrimp of the U.S. National Research has proposed that D.P.:D.E. is a more rational and precise way of conveying crude protein requirements in fish feed. This ratio is important in aquaculture production because a correct ratio will lead to the most efficient conversion of dietary protein into body mass, which maximizes growth and minimizes feed consumption.

Methionine Requirements Of Adult Fish

Work by Jonathan Goff and Delbert Gatlin III found that methionine is one of the first limiting essential amino acids in many fish diets, especially those containing higher levels of plant protein sources such as soybean meal, peanut meal and copra meal. With substitution for fishmeal in diets, limiting components such as the sulfur amino acid methionine need to be supplemented.

Many investigators have reported the quantitative methionine requirements of commonly cultured fish species as ranging 18-40 g/kg. This year, J. Niu and co-authors reported the requirement for methionine in golden pompano, *Trachinotus ovatus*, as 10.6 g/kg diet. These results were valuable, but the study was performed on small fish with initial body weights of 12.5 g and final body weights of 72.0 g.

Larger cobia performed well on a diet with the majority of fishmeal replaced by soy.

The authors' study focused on determining the methionine requirements for large Florida pompano, *Trachinotus carolinus*, with initial weights of 208 g and final weights of 505 g. These larger fish naturally consumed higher amounts of food. The first results of the investigation suggested that Florida pompano adults perform well as far as growth and feed efficiency with values of methionine below those published by J. Niu.

Perspectives

A closer look at the nutritional requirements and digestive capacity of larger fish is necessary to optimize growth and minimize waste in commercial marine fish farms. These mammoth goals can be more rapidly attained under synergistic collaborations among all stakeholders in the production chain, from suppliers of raw materials and aquafeed manufacturers to commercial farmers and research institutions.

Biomass Density Affects Bass Production, Not Feed Conversion, Growth Rates In RAS

Arkansas is the leading producer of food-size and fingerling largemouth bass in the United States. Photo by Scott Jones.

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Summary:
The years-long production period for largemouth bass in ponds hampers the economic feasibility of pond culture in the United States. The use of a recirculating system would allow temperature control during colder months and make year-round bass production feasible. A study demonstrated that bass fingerlings could be raised at high densities in a semi-closed recirculating system without serious mortality, size variability or growth retardation. Although the ranges of size distribution were similar among treatments, more fingerlings were near the average size at lower biomass densities.

In recent years, interest in the production of largemouth bass, *Micropterus salmoides*, has steadily increased in the United States. However, the current production systems utilized by the commercial aquaculture industry in Arkansas, the top U.S. bass-farming state, create an extended culture period of two to three years, which tends to limit the economic feasibility of food fish production of largemouth bass.

Spawning techniques and pond culture practices for largemouth bass have been well established since the 1960s. Farmers typically rely on natural spawning in the early spring, rearing fry in nursery ponds, feed training small fingerlings in indoor tanks and restocking larger feed-trained fingerlings to ponds. While this method can be effective, it has some drawbacks. One key issue involves the reintroduction of feed-trained fingerlings to growout ponds in the middle of the summer, which can result in poor survival due to factors such as water quality, disease and bird predation.

Alternative: Recirculation Systems

Researchers in Florida, USA, recently reported the successful production of advanced bass fingerlings in the fall and early winter months using controlled spawning techniques. Utilizing these in Arkansas, which has much lower winter temperatures, could be problematic for commercial producers.

To sustainably produce largemouth bass and shorten the overall culture period for the food fish market, commercial producers will need to rely on new culture techniques. The use of a recirculating system would allow control of culture temperature during the winter months, making year-round production of largemouth bass feasible.

Largemouth bass fingerlings were stocked at experimental biomass densities of 15, 20, 25, 30, 35 and 40 kg/m^3.

Biomass Control

Biomass density is a critical husbandry factor in closed or semi-closed recirculating systems. Existing aquaculture production strategies often manipulate biomass density by harvesting, grading and transferring to increase production.

The effects of biomass density can be species-specific and also depend on social interactions at very low and/or very high biomass densities. In predatory fish such as largemouth bass, biomass density may need to be adjusted for cannibalism. While there is some data in the literature on pond production techniques, little research has been conducted on biomass densities of largemouth bass raised in recirculating systems.

RAS Study

The authors designed a study to evaluate a culture paradigm for producing advanced largemouth bass during the winter using feed-trained bass fingerlings in a semi-closed recirculating system. This trial examined the effects of biomass density on the size variability and growth performance of the fingerlings.

During the winter of 2013, feed-trained largemouth bass fingerlings of 9.04 g initial weight were secured from Dunn's Fish Farm in Brinkley, Arkansas, and transported to the Aquaculture Research Station at the University of Arkansas at Pine Bluff. The semi-recirculating experimental system, which had a total water volume of 3,500 L, consisted of 18 square plastic tanks, a radial flow settler, a sump, a moving-bed filter, a centrifugal pump, a sand filter, a downflow oxygen saturator and an ultraviolet sterilizer. The daily water exchange rate was 30 to 50%.

The fingerlings were stocked at experimental biomass densities of 15, 20, 25, 30, 35 and 40 kg/m^3 (treatments T1, T2, T3, T4, T5 and T6) with three replicate tanks per density. Water temperature was main-

Table 1. Growth performance of largemouth bass fingerlings with different biomass densities in a semi-closed recirculating system.

Treatment	15 kg/m³	20 kg/m³	25 kg/m³	30 kg/m³	35 kg/m³	40 kg/m³
Final weight (kg)	8.6^{d}	11.6^{c}	13.1^{c}	16.9^{b}	17.9^{b}	20.9^{a}
Gross yield (kg/m³)	51.2^{d}	70.0^{c}	78.8^{c}	101.5^{b}	107.6^{b}	125.6^{a}
Feed-conversion ratio	0.96	0.94	1.08	0.99	1.13	1.10
Specific growth rate (%/day)	2.03	2.07	1.90	2.01	1.85	1.89
Survival (%)	95.0^{b}	96.1^{ab}	96.4^{ab}	96.2^{ab}	97.4^{a}	96.8^{a}

Values with the same superscript within the same row are not significantly different, $P < 0.05$.

Table 2. Size variability of largemouth bass fingerlings with different stocking densities in a semi-closed recirculating system.

Treatment	15 kg/m³	20 kg/m³	25 kg/m³	30 kg/m³	35 kg/m³	40 kg/m³
Final individual length (cm)	13.1	13.2	12.8	13.0	12.6	12.8
Final individual weight (g)	28.7	29.8	27.3	28.6	26.9	28.1
Condition factor	1.24	1.26	1.25	1.24	1.28	1.31
C.V. – total body length (%)	8.7	10.0	11.4	10.9	11.2	11.1
C.V. – body weight (%)	26.9	32.4	36.1	36.6	36.2	34.4
C.V. – condition factor (%)	8.5	8.3	8.9	8.2	8.1	9.2

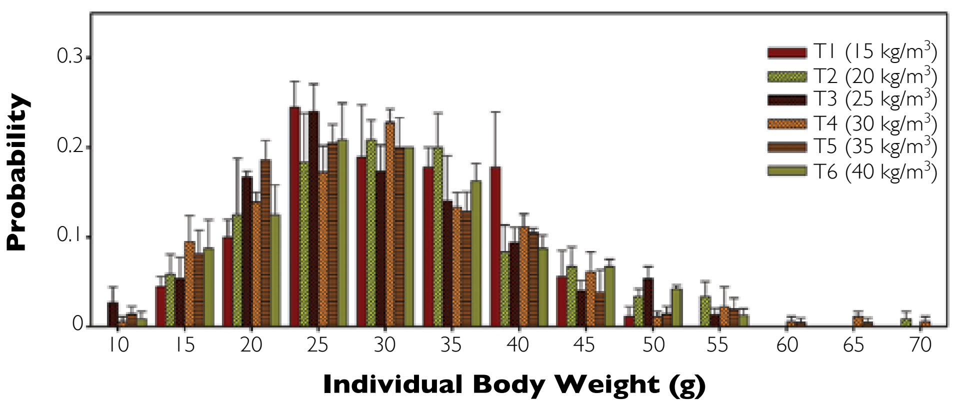

Figure 1. Distribution of individual body weights of largemouth bass fingerlings at different stocking densities.

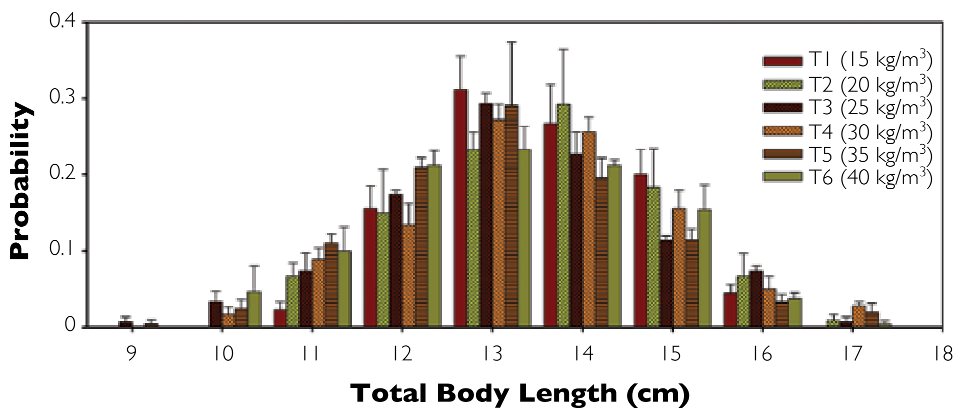

Figure 2. Distribution of total body lengths of largemouth bass fingerlings at different stocking densities.

tained using submersible heaters. Gentle aeration was installed in the middle of each tank for mixing water and efficient solids removal toward the center drain.

The fish were fed a formulated diet with 42% protein and 16% lipid, distributed by automatic feeders from 9 a.m. to 9 p.m. The daily feed ration was initially set at 5% of total body weight and readjusted to the initial body weight weekly. Mortality was checked twice daily.

After 60 days, feed-conversion ratios, specific growth rates and survival rates were determined. Ten percent of the total population in each tank was randomly sampled to measure individual body weight and length. Based on individual measurements, coefficients of variation (C.V.) were calculated to evaluate the effects of biomass density on size variability.

Results

The water temperature was stable at 25.4 ± 1.6° C, and dissolved-oxygen concentrations remained above saturation for all treatments. pH and alkalinity were 6.93 ± 0.26 and 53.3 ± 8.7 mg calcium carbonate/L, respectively. The average total ammonia nitrogen (TAN) and nitrite-nitrogen concentrations were 1.88 ± 0.62 and 0.67 ± 0.48 mg/L, respectively. The TAN concentrations in the T4, T5 and T6 treatments were significantly higher than those in T1 and T2.

Survival ranged between 95.0 and 97.4% across all treatments (Table 1). The survival rates in the two highest biomass densities (35 and 40 kg/m³) were significantly higher than at the 15 kg/m³ density, but were not different from the rates under the 20, 25 and 30 kg/m³ treatments. Biomass density did not appear to affect the feed conversion and specific growth rates of the largemouth bass fingerlings. Biomass density significantly affected production rates, with production ranging 51.2-125.6 kg/m³.

Coefficients of variation in total body length, individual weight and condition factor at the end of the experiment did not show any differences among treatments, but uniformities for those parameters tended to be lower in the highest biomass density (Table 2). Distributions of total body weight and total length by treatment are shown in Figures 1 and 2.

Perspectives

Intensification is vital for maximizing fish production within a given culture system. However, in some cases, high biomass density can result in profit loss due to growth reduction, high mortality, impaired feed conversion or a high level of size variability. To avoid such problems, the level of intensification for largemouth bass should consider the production phase.

In an intensive system, determining optimal biomass density is a compromise process that maximizes yield, minimizes individual growth reduction and maintains an adequate culture environment. In fingerling production, size variability is likely another important factor, since greater size variation can reduce the efficiency of nursery management and ultimately extend the culture period.

A biomass density of 100-125 kg/m³ from the initial biomass density of 30-40 kg/m³ may be attainable for largemouth bass up to 30 g in weight reared in a semi-closed recirculating system.

The feeding demonstration at Amazon Fish Products S.A. evaluated the performance of paiche given three different feeds.

Soy-Based Feeds Evaluated For Production Of Fast-Growing Amazonian Paiche

Summary:
A cooperative feeding demonstration conducted by the U.S. Soybean Export Council and Amazon Fish Products S.A. in Peru evaluated the use of soybean meal and soy protein concentrate to partially replace fishmeal in diets for paiche. Both soy products have high protein and low oligosaccharide content with minimal anti-nutritional factors. Results showed that inclusion of the soy products led to fish performance similar to that achieved with a fishmeal-based diet. Due to variables in test protocols, the results of this demonstration should be considered anecdotal.

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Arapaima gigas, also known as arapaima, pirarucu or paiche, is the largest freshwater fish. Growing up to 3 m in length and 200 kg in weight, they are endemic to tropical South America and are found primarily in the lowlands of the watersheds of the Araguaia-Tocantins, the Solimões-Amazon River, and Essequibo River basins in Brazil, Guyana and Peru.

Although the paiche has been included on the international trade-restricted list due to the fact that it is considered endangered by the Convention on International Trade in Endangered Species, the company Amazon Fish Products S.A. has a permit to commercialize the fish because they have been produced by aquaculture since 2009. The interest in growing paiche is related to the fact that the fish have the best growth rate among Amazonian cultivated fish species, growing 27-41 g/day and up to 15 kg/year.

Paiche Diets

Paiche feed on prey consisting primarily of detritivorous/algivorous and omnivorous fish that are not top predators, and probably play a key role in regulating the energy and nutrient flows of aquatic ecosystems. In aquaculture, the fish are fed high-protein (45%) feed, which can become a limiting factor for production due to the cost and availability of fishmeal ingredients.

The authors therefore performed a commercial feeding demonstration to evaluate the use of new soy ingredients that have high protein content and low levels of the oligosaccharides raffinose and stachyose as partial replacements for fishmeal in diets for paiche. The feeding demonstration was conducted at the Amazon Fish Products hatchery in Ucayali, Pucallpa, Peru.

Study Setup

Three test diets formulated to contain 45% crude protein and 13% fat were assigned to two 0.85-m^3 replicate tanks in sequential order, with a commercial diet (COM) in tanks C1 and C2, an experimental diet with low-oligosaccharide soybean meal (S.M.) in tanks C3 and C4, and an experimental diet with soy protein concentrate (SPC) in tanks C5 and C6. All tanks were operated with a constant water flow of 5 L/minute.

Fish for the demonstration had an average individual weight of 647.4 g. They were intended to be stocked at a density of 62 fish/tank, with an average fish biomass equivalent to 47.4 kg/m^3, but some of the fish jumped between tanks. The results of this demonstration should therefore be considered anecdotal.

The fish were fed ad libitum, and six fish were sampled weekly from each tank over a 62-day period. Individual fish weights and lengths were determined during each sampling. Fish were harvested after 62 days at an average weight of 2.0 kg.

Results

The weekly growth data is shown in Tables 1 and 2. Final average weights for fish fed the commercial diet were 2,198 and 2,132 g in each replicate tank, while average weights for fish fed the S.M. diet were 2,121 and 2,166 g. Average fish weights for the SPC treatment were 1,981 and 2,098 g.

Interest in growing paiche relates to the fact the fish can grow up to 15 kg/year in culture.

Table 1. Production data collected after a 62-day feeding demonstration with paiche.

Treatment	Replicate	Number of Fish Initial	Number of Fish Final	Survival (%)	Individual Weight (g) Initial	Individual Weight (g) Final
COM	C1	61	61	100.0	647.44	2,197.7
	C2	64	64	100.0	647.44	2,132.0
S.M.	C3	53	52	98.1	647.44	2,120.8
	C4	60	60	100.0	647.44	2,166.0
SPC	C5	62	55	88.7	647.44	1,980.8
	C6	75	74	98.7	647.44	2,097.8

Table 2. Production data collected after a 62-day feeding demonstration with paiche.

Treatment	Replicate	Weight Gain (%)	Specific Growth Rate	Biomass Gained (kg)	Final Density (kg/m^3)	Feed-Conversion Ratio
COM	C1	239.43	1.97	94.56	158.27	0.92
	C2	229.30	1.92	95.01	161.10	0.87
S.M.	C3	227.57	1.91	75.97	130.20	0.99
	C4	234.55	1.95	91.11	153.44	0.92
SPC	C5	205.94	1.80	68.80	128.62	1.18
	C6	224.01	1.90	106.68	183.28	0.94

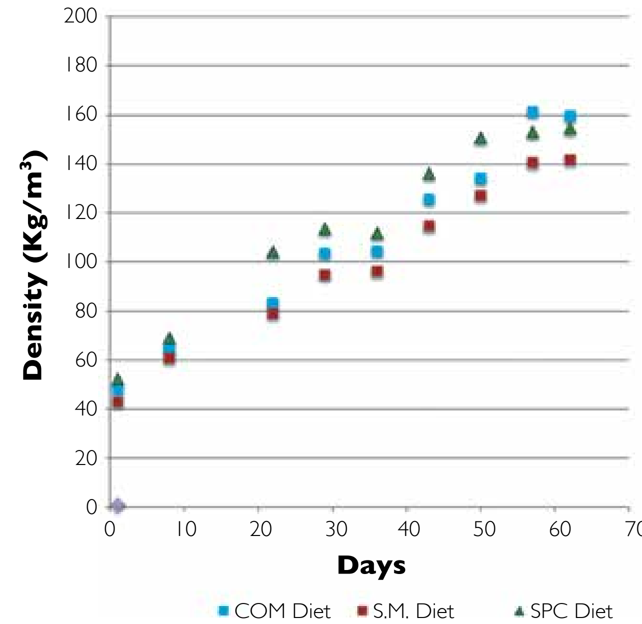


Figure 1. Average culture density for paiche given three test feeds.

Relative weight, a measure of fish condition, indicates if animals are thin or fat. A fish with a relative weight value of 80% or less is considered severely thin, indicating a lack of food. Fish for this demonstration were in very poor condition at the time of stocking, with relative weights of 78.59 ± 4.09%. After the first sampling, fish condition improved to an average of 94.57%, and by the end of the demonstration, the average relative weight for all the fish was 99.07%.

Fish biomasses at harvest were 158.3 and 161.1 kg/m^3 for the two tanks fed the commercial diet. Fish in tanks fed the soybean meal-based diet had harvest biomasses of 130.2 and 153.4 kg/m^3, while fish fed the SPC diet had biomasses of 128.6 and 180.8 kg/m^3 (Table 2, Figure 1).

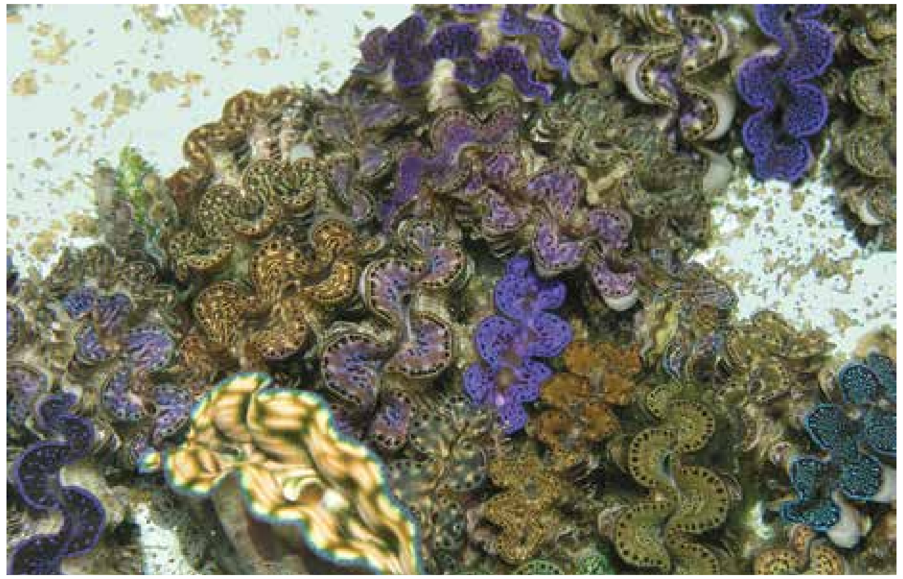
With a value of 234.4%, average weight gain was highest for paiche given the commercial diet. Fish that received the S.M. feed had weight gains of 231.1%, and paiche on the SPC diet gained 215.0%.

Feed-conversion ratios (FCRs) were lower for fish fed the COM diet, with an average ratio of 0.895 ± 0.035. Fish fed diet S.M. had an average FCR of 0.950 ± 0.057, and fish fed the SPC diet had an average FCR of 1.060 ± 0.170.

Perspectives

The results of this study confirmed that paiche can be raised at very high densities. Diets based on soybean meal and feed-grade soy protein concentrate yielded fish performance similar to that for paiche fed a fishmeal-based diet. Hence, soy-based feeds can be alternatives to traditional fishmeal-based feeds for the continued development of a commercial paiche-farming industry.

Community-Based Approach Supports Aquaculture Development In Pacific Islands



In the Marshall Islands, giant clams are harvested for local consumption or grown for aquarium trade as a means to increase community/individual income.

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Summary:
Threats from overfishing and climate change highlight the need for alternative methods of local seafood production and economic advancement throughout the Pacific Islands. Together with industry partners, the Center for Tropical and Subtropical Aquaculture emphasizes the importance of developing aquaculture in a way that preserves the unspoiled environments and honors the unique community-focused traditions of this region. Ongoing work with giant clams, sponges, pearl oysters, sea cucumbers and crabs has resulted in production that helps meet consumer needs as well as replenish natural stocks.

The Pacific Islands are a vast, remote region home to pristine ecosystems and environmental conditions that are ideal for aquaculture. While there has been some localized industry development, the isolation of the region presents distinct challenges and opportunities.

In contrast to the mainland, where land space and capacity to produce large quantities of food are ample, farming resources in the Pacific region are limited, and large-scale development is unlikely. Thus, regional industry stakeholders must look for innovative ways to make a positive impact on the future security of the local seafood supply.

Regional Aquaculture Centers

Fish is a staple protein for most Pacific Islanders, and fishing is an integral part of many community traditions and local economies. However, increasing threats from overfishing and climate change highlight the serious need for alternative methods of local seafood production and economic advancement throughout the region. To address this issue, multiple institutions and federally funded programs have come together to spearhead aquaculture efforts in Pacific Island communities.

The Regional Aquaculture Center Program was established by the United States Department of Agriculture in 1986 to integrate individual and institutional expertise and resources in support of commercial aquaculture development. Its Center for Tropical and Subtropical Aquaculture (CTSA) assists aquaculture development in Hawaii and the U.S.-affiliated Pacific Islands, with annual funding support for various research, demonstration and extension projects.

Together with industry partners, CTSA emphasizes the importance of developing aquaculture in a way that preserves the unspoiled environments and honors the unique traditions of this region. Unlike most other places in modern society, the Pacific Islands are home to a palpable history of community-based living. While each community is different, most are steeped in customs of working together for the benefit of everyone.

Resource Management

Elaborate resource management systems like the Hawaiian "ahupua'a," a complex system of land division used by ancient Hawaiians, were dependent on the collective labor of the community to gather and produce food. Currently, community farming has mostly fallen by the wayside. The overall sense of community throughout the region, however, is still very strong. This "foundation of livelihood" has been a largely untapped resource in modern development efforts, yet holds the key to the most effective way to achieve positive impacts from aquaculture in the region.

This "foundation of livelihood" holds the key to the most effective way to achieve positive impacts from aquaculture in the region.

Extension agent Masahiro Ito trains local technician Belenko Halverson in pearl grafting in Pohnpei, Micronesia.

Employing an innovative community-based approach to new development, coupled with guidance via technical assistance, is helping islands reclaim the food and economic independence that were once a defining part of their identity. In Hawaii, there has been a recent resurgence in traditional community-based food production, spurred by the restoration of several ancient fish ponds.

Aquaculture In Hawaii

Hawaii has a rich aquaculture history dating back centuries ago, to when communities maintained and harvested a variety of fish and shellfish from large coastal fish ponds called "loko i'a." Publicly funded organizations such as U.S. National Oceanic and Atmospheric Administration (NOAA) fisheries and CTSA have been working together with Kua'āina Ulu 'Auamo (KUA), a grassroots non-governmental organization, to restore these fish ponds throughout the Hawaiian Islands.

Through an organized network managed by KUA, pond operators share their community work forces to help each other repair damage to ponds. While the NOAA Pacific Island Regional Office is working to streamline the permitting process for this physical restoration of the ponds, CTSA is working with researchers throughout the state to restore the biological knowledge for raising fish.

For example, despite having ideal growout conditions, local shellfish production halted for decades through the end of the 20th century, until a dedicated group of stakeholders reestablished a local bivalve aquaculture industry in 2012 to reduce dependence on the roughly 400,000 oysters currently imported into the state each month. Other targeted species for aquaculture production in fish ponds have included seaweed, "live rocks," feather duster, mullet, milkfish and moi.

Development Plans

With the implementation of well-conceived, efficient plans for development, more Pacific Islands can capitalize on the willingness for community participation in aquaculture production in the same way Hawaiians embrace it. Knowing the opportunities and challenges of the isolated region, farming efforts to this point have largely focused on non-fed production.

In the Republic of the Marshall Islands, Marshallese are being trained in hatchery techniques for giant clams and are releasing the animals into community-managed open water areas. The clams are either harvested for local consumption or grown to the desired size for aquarium trade as a means to increase community/individual income.

In the Federated States of Micronesia, bath sponge-farming technology has been transferred to many villages for community growing efforts. Pearl oyster seeds have also been distributed to villages to culture and eventually harvest for profit.

In addition, there has been a recent focus on sea cucumber production. Foreign commercial fishing operations have overharvested high-value sea cucumbers to the point of near extinction, particularly in the Western Pacific. Excessive removal of the filter-feeding species can have a serious impact on the ecological balance of nearshore ecosystems. Sea cucumber work has helped to address this issue by first developing and refining simple hatchery and growout techniques, and then providing hands-on training for Micronesians.

In nearby Palau, aside from the introduction of giant clam hatchery technology, researchers have successfully established a mangrove crab hatchery to supply crablets to local farmers, and are now working to establish a rabbitfish and milkfish hatchery. Resulting production continues to help meet consumer needs as well as replenish natural stocks in lagoons throughout the region.

All of the aforementioned activities have the potential to be of great ecological and economic value for the region. Furthermore, community farming efforts have had positive results in building skills, improving livelihoods and bringing communities together.

Perspectives

Hawaii and the U.S.-affiliated Pacific Islands have an opportunity to demonstrate an innovative method of aquaculture farming – one that does not emphasize producing mass quantities of food for export, but rather focuses on meeting the needs of the immediate community. A farming system where everyone has a vested interest and subsequently takes pride in their roles.

It has been suggested that small- and medium-scale community-based farming will be an important aspect of solving world hunger. The Pacific Islands region is the perfect place to show how communities can work together to meet their own food security needs, limit their dependence on imported goods and relieve fishing pressure on wild stocks.

A young girl from Pohnpei proudly displays her community's pearl harvest.

With the implementation of well-conceived, efficient plans for development, more Pacific Islands can capitalize on the willingness for community participation in aquaculture production.

Russian Food Embargo: Who Have Been Hurt?

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Fresh fish at this Moscow hypermarket seafood counter and elsewhere in Russia are reflecting higher prices due to the embargo. Photo by Tatjaja Feodoritova, Norwegian Seafood Council.

Summary:
Russia's 2014 embargo on seafood and other food imports from the United States, European Union members and other countries created multiple impacts. Russian consumers have experienced higher prices and declines in the volume and quality of seafood available. Facing shortages in raw materials, Russian companies in seafood value chains have also been hurt. The largest import decreases have been for salmon and trout, but herring, mackerel and capelin imports have also dropped. Norwegian products have been replaced by salmon from other countries.

In August 2014, Russia introduced an embargo on food imports, including seafood, from the United States, all European Union member states, Australia, Canada and Norway. After 14 months, what have been the consequences of the embargo? Consider the impacts on the global market for farmed salmonids – salmon and ocean-farmed trout.

More-Diversified Market

During the same time period, the salmon market has grown in terms of the number of importing countries, leading to a geographically less-concentrated global market. This is illustrated by the development of Norwegian exports of farmed salmonids, as measured by the Herfindahl market concentration index (Figure 1).

The index is defined as the sum of the squared import share of each import country. If all exports were destined for a single export market, the Herfindahl index value would be 1, while if the exports were distributed among a large number of importing countries, the Herfindahl index would approach 0.

Figure 1 shows the Herfindahl index for Norwegian salmonid exports steadily declined from 1990 to 2014, implying that the dependence on individual import countries has also declined over time. Norway exported salmonids to around 100 countries in 2014.

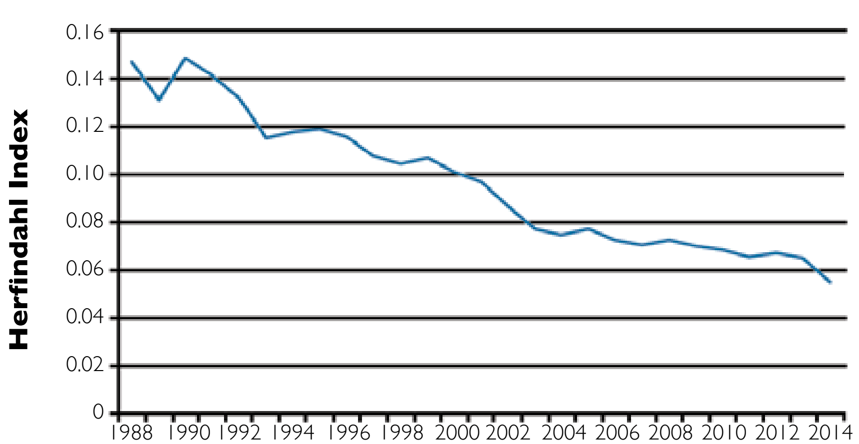


Figure 1. Export market concentration for Norwegian farmed salmonids.

Russian Food Embargo

On August 7, 2014, the Russian government issued a decision that defined the banned food products and the source countries concerned. The list of banned products included meat, fish, milk and dairy products, fruits and vegetables. The embargo included most of the products in the Harmonized Tariff Schedule category for fish. The list was modified by an August 20 government decision that made an exception for salmon smolts, which can be used for domestic salmon farming.

In June 2015, the embargo was extended to August 6, 2016. On August 13, Russia also banned products from Albania, Iceland, Liechtenstein, Montenegro and Ukraine.

Before the embargo, Russian imports of fish increased year by year and reached a value of U.S. $3.2 billion in 2013 for 1.03 mmt of fish and seafood. When the food embargo was introduced, imports declined. For the first six months of 2015, the import volume was down 53%. Figure 2 shows the declines in monthly import volume and value since August 2014.

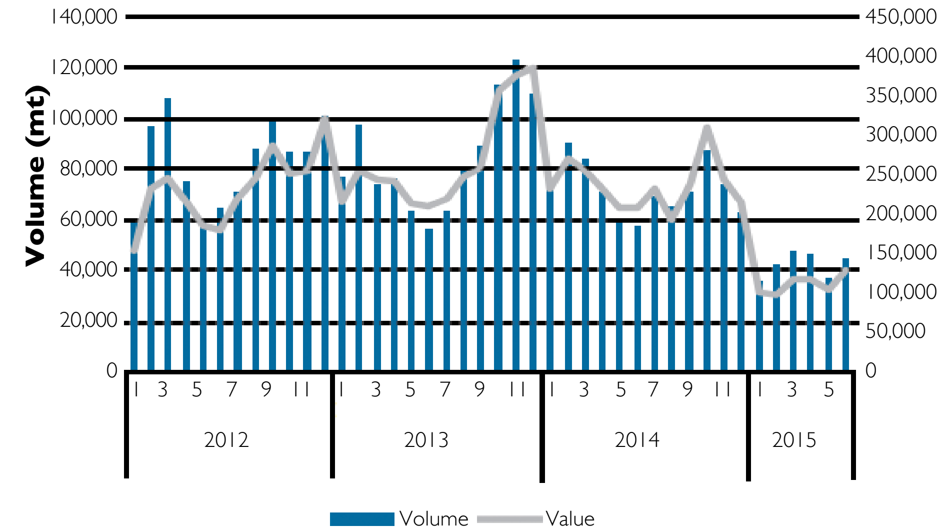


Figure 2. Monthly Russian imports of fish and seafood products.

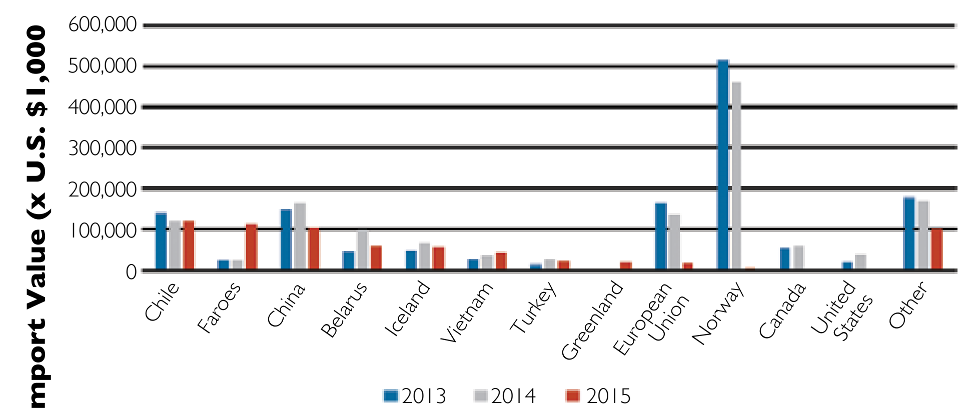


Figure 3. Value of Russian imports of fish and seafood products.

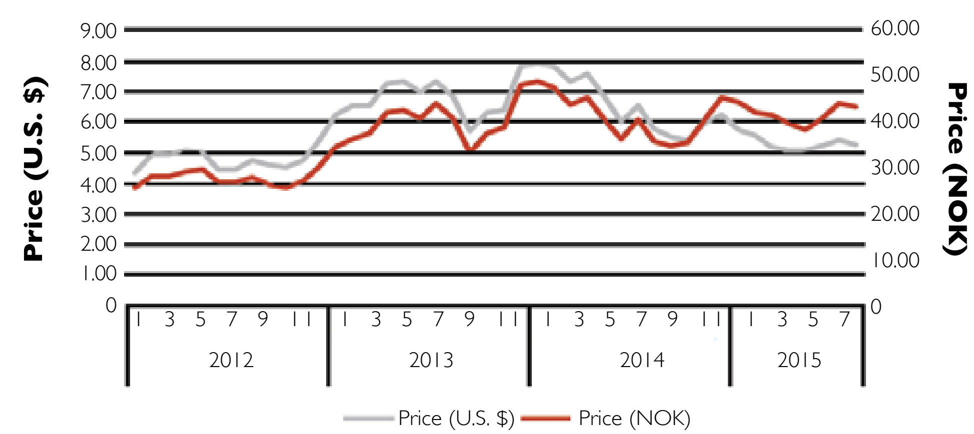


Figure 4. Monthly prices for Norwegian head-on gutted salmon.

When the food embargo was introduced, imports declined. For the first six months of 2015, the import volume was down 53%.

The effects of the embargo on imports from different export countries and regions are shown in Figure 3. Imports from Norway, Canada, the U.S. and E.U. have virtually disappeared.

Import Substitution

In the same period, Russian fish catches increased by approximately 150,000 mt. The catches consisted to a large extent of Alaska pollock and pelagic species, with mackerel the most important. Russian exports of fish in the first half of 2015 decreased by approximately 80,000 mt, mostly Alaska pollock and herring.

The largest import decreases in value have been for salmon and trout, but herring, mackerel and capelin imports have also decreased substantially. In total, the supply of fish in the Russian market seems not to have been greatly affected, but along with an increase in Alaska pollock has come likely decreases in most other important species.

Russian imports during the last years before the food embargo consisted of more than 200,000 mt of salmon and trout – mainly from Norway – and more than 400,000 mt of pelagic fish species that came mainly from Norway and E.U. countries. Some of the pelagic fish have been replaced by fish from Iceland and the Faroes, but the import volumes are approximately half of what they used to be.

Salmonid Imports

It is difficult to substitute similar products for salmon and trout, especially on a short-term basis. Norwegian products have been replaced by salmon from other countries to some extent. The Faroe Islands have increased their exports of mostly fresh, whole salmon to Russia to 9,000 mt. Chile has also increased its exports, with 7,000 mt of frozen, whole salmon.

In comparison, during the first half of 2014, Russia imported 41,000 mt of salmon and 11,000 mt of trout from Norway. In 2015, only some live salmon for breeding have been imported from Norway.

Almost half of the imported salmon usually goes into salting and smoking, and there is evidence this share has been more or less stable through this period. Salmon and trout have also been very popular in restaurants, especially as main ingredients in the many sushi restaurants all over Russia.

Consumers, Industry Affected

The Russian food embargo has led to an increased scarcity of fish in the Russian market. Both the industry, from importers to small producers, and consumers have been affected. Since the embargo, many middle-class consumers have experienced lower purchasing power. Consequently, they have not been able to consume more-expensive import products in the same amounts as before.

The Russian fish market has been severely impacted by the lack of fish as raw material for the processing sector, resulting in the suspension of activity or even closure of several processing companies. The overall decline of the Russian economy – caused by falling oil prices and the weakening of the Russian ruble – has contributed to declining domestic demand for seafood products.

The Russian seafood sector is currently characterized by concern over declining consumer purchasing power and the ability of businesses to maintain their activities in a situation with a weak ruble and a sharp increase in key interest rates.

Resilient Salmon Market

As described earlier, the global salmon market has both grown in volume and become geographically more diversified. This has made the market more robust to shocks from individual import countries.

As shown in Figure 4, it is hard to detect any effect on salmon prices from the Russian food embargo after August 2014. Russia represented around 13% of Norwegian salmonid exports in 2013, and was Norway's largest export market. The embargo led to a reduction to a 6% export share in 2014, and then to virtually zero in 2015.

Although significant short-term costs were inflicted on some individual companies with large shares of production going to Russia, the Norwegian industry as a whole was able to shift its production to other markets with limited costs. Other salmon-producing countries not affected by the embargo have increased their exports to Russia, to some extent by reducing their exports to other markets.

Who Have Been Hurt?

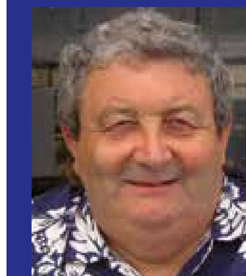
So far, the biggest losers from the Russian food embargo have been Russian consumers. They have experienced a decline in the volume of seafood available to them, a reduction in the quality of several seafood products and higher prices. Russian companies in seafood value chains have also been hurt, particularly those highly dependent on established relationships with suppliers in banned countries.

Individual companies in the countries excluded from exporting to Russia have also incurred significant costs from the loss of the Russian market. But by and large, companies in exporting countries have been able to shift to other markets. The salmon industry, in particular, has demonstrated that it is globally diversified, and that even the closing of one of the largest national markets has limited effects.

Russian consumers have experienced a decline in the volume of seafood available to them, a reduction in the quality of several seafood products and higher prices. Russian companies in seafood value chains have also been hurt.

Abundant Food, Yet People Are Starving

Fish fit well in the popular Paleo diet, but the Paleo approach may not work well for all consumers.



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Summary:
While significant numbers of people are undernourished, an increasing number of people are overweight. Experts continue to raise these and related issues. Global average meat consumption has nearly tripled in 50 years, but people are increasingly disconnected from its production. Farmers have become part of a world driven by companies that respond to shareholder values. Food system problems can no longer be only for health authorities to solve. The divide between recommendations and results is marked, so we must learn more about food production and change our behaviors.

How can we have 800 million starving people and at the same time have obesity in over one-third of the global population? We have sufficient food. We have nutritious food. Yet malnutrition and obesity are affecting people globally.

When World War II concluded in 1945, there was much food scarcity, but since then, the world has changed. Of course, there are still pockets of shortages, but generally speaking, we have moved away from food shortages. Technology and innovation in production have improved crops and yields immensely.

People of the Pacific nations, for example, are suffering from obesity. More than 30% of Fijians are obese, and, amazingly, American Samoan women have an obesity rate as high as 80%. Even in Indonesia, and in fact many developing countries, there are more overweight than underweight women. That effect is seeing a doubling of the burden of disease.

With significant numbers of people undernourished and an increasing number of people overweight, specialists are saying surely something is not right with our system. While there are theories on how to solve the issues, no one has really found a workable solution. Health departments have failed fairly dismally, and this has resulted in more issues, with non-communicable diseases such as diabetes taking limbs and lives along the way.

Sydney Conference

The "Resetting the Australian Table: Adding Value and Adding Health" conference recently held in Sydney, Australia, engaged a number of specialists who not only raised the issues, but endeavored to find solutions to the mounting problems.

Professor Jonathan Rushton from the Royal Veterinary College at the University of London was quoted as saying "Just as farmers care about what they feed their animals, they should care about how their food is processed."

Rushton said annual global average meat consumption has nearly tripled in 50 years – from 25 to 62 kg/person – and people are increasingly disconnected from its production. However, meat consumption hasn't changed a great deal in Australia. In the 1960s, annual consumption was 100-110 kg/capita. Consumers have shifted form eating sheep and cattle to eating more pigs and poultry.

"When I travelled between Melbourne and Sydney, what I didn't see was pigs and poultry out in the fields," he said. "Those systems are hidden from you. You don't see what they're being fed. There is a disconnect between the urbanized population and food systems."

Rushton described the food system as rotten, and said farmers have become part of a world driven by stock-listed companies that respond to shareholder values.

Changing Behavior

At the same event, Associate Professor Robyn Alders of the Charles Perkins Centre and Faculty of Veterinary Science at the University of Sydney suggested that food system problems can no longer be only for health authorities to solve, but that the whole of society needs to engage and assist with solutions.

Alders, who has long worked in international agricultural aid in Africa and Asia, reportedly said: "If we feed our pigs ad lib they will get too fat to stand up, but we are doing that to ourselves. You have ongoing significant numbers of people who are undernourished and an increasing and significant number of people who are overweight."

Sociologist Dr. Jane Dixon of the Australian National University explained that her doctorate work looked into the rise of chicken in our diets, saying it met all the supermarket criteria as convenient, cheap and offering choice.

Dixon said chicken has been marketed as a healthy, low-fat meat, winning the National Heart Foundation's tick of approval. Australian consumption of chicken has doubled to 43 kg/person/year. She told the conference that "we are contradictions" and are buying the wrong chicken, highlighting that when coated in batter and deep fried, chicken's goodness is undone.

Paleo Diet

The "Paleo diet" has been taking the world by storm. An industry has been created where there was none. Books, magazines, blogs, podcasts and retreats have been spreading the idea that eating what cavemen ate – lots of meat, fish and plants; no grains, sugar and dairy products – is the path to weight loss and better health.

Most cavemen died by the age of 25 but, of course, things were different back then. High infant mortality was a dramatic contributor to this pattern, but the fact remains that few made it anywhere close to the modern life expectancy of 75 to 80 in western countries.

The divide between recommendations and results is marked, but not all that uncommon, be it about a diet or a business. In a world seemingly overrun with data, analysis and experts, what people say we should do and what actually works can be like ships passing in the night.

Perspectives

The solutions we seek must be responsible and sustainable. We must seek hard evidence and promote those facts. The Paleo diet sounds intriguing – and parts of it might even be right – but it makes sense to see how it actually does in practice. It may or may not be for you.

Aquaculture will be front and center at the GOHWell Summit – February 4-6, 2016 in Melbourne, Australia. Fish feeds and diets will be discussed in detail. It is important that we do not fall into the same trap as pork and chicken.

Human Enteric Viruses in Shellfish

Part IV. Norovirus Prevalence In Europe

The presence of noroviruses in oysters varied significantly in different countries.

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Summary:
Pathogenic noroviruses have been identified in shellfish from various countries in Europe. In long-term studies in Italy, for example, the average positivity for norovirus in shellfish was 4.1%. In a shorter-term study in France, about 9.0% of the sampled oysters were contaminated with norovirus. In Ireland, norovirus GII.4 was responsible for the majority of outbreaks, but multiple genotypes were identified in oysters during a two-year study. In research on multiple species in Portugal, viral contamination was detected throughout the year in several shellfish species and all collected areas.

As reported in previous articles in this series, noroviruses are the major cause of outbreaks of acute gastroenteritis in humans. Consumption of shellfish is one of three main transmission routes of norovirus infection.

This article will discuss the viruses' presence and significance in shellfish produced in various countries in Europe. It is important to note that some molluscan shellfish in the study reporting were obtained from prohibited areas, areas known as receiving untreated sewage or from growing areas classified as B and C by the European Union Commission. Therefore, some of the studies may not reflect the potential for human illnesses due to harvest restrictions for direct human consumption.

Italy, Various Species

A study in Italy carried out from 2003 to 2011 was initiated to define the prevalence of norovirus contamination in 4,463 samples collected from commercial shellfish production areas designated for direct human consumption. Samples included 2,310 mussels (*Mytilus galloprovincialis, M. edulis*); 1,517 clams (*Tapes* species, *Callista chione*); 510 oysters (*Crasssotrea gigas, Ostrea edulis*); 22 minor species (*Ensis* and *Murex* species, *Pecten jacobaeus, Tellina* species, *Agropecten purpunratus, Chamelea gallina, Glycineris* species and *Venus verrucosa*) and 104 samples of preserved fish salads, fish fillets, fresh or frozen fish, squid, shrimp and prawns.

Among the samples analyzed, 86.7% were domestic, and 13.3% were imported. About half of the samples were collected in the Veneto region on the Adriatic Sea, while the remainder was collected in Emilia-Romagna, Liguria and other major coastal regions: Marche, Puglia, Campania, Sardinia and Sicily.

The average positivity rate for norovirus presence was 4.1% and ranged from 0.6% in 2007 to 9.8% in 2003. The values ranged from 1.9% in preserved products to 4.7% in mussels. Genetic characterization showed a prevalence of genogroup II genotypes, including GII.b, GII.e and different GII.4 variants.

Switzerland, Oysters

To assess the percentage of virus-contaminated *Crassostrea gigas* and *Ostrea edulis* oysters imported into Switzerland, 87 samples consisting of five oysters each were analyzed for the presence of noroviruses from November 2001 to February 2002.

Sixty-one of the samples were exported by 31 different French suppliers, 12 of the samples were exported by three

Dutch suppliers, and 14 of the samples were exported by two Irish suppliers. Eight of the 87 samples from six of the 31 French suppliers were positive for noroviruses.

France, Oysters

A total of 387 oyster samples harvested in France between February 2010 and May 2011 were collected from 42 different locations based on market availability. Among the samples, 149 were produced on the southwestern coast, and 72 came from the western coast at Brittany-Normandy. The production locale for the remaining 167 samples (in supermarkets) could not be identified due to information contained on the sanitation label. Among the 387 samples, 91% were norovirus-negative, and 9% were contaminated with norovirus (Table 1).

One hundred five samples were produced in higher-quality areas with less than 230 *Escherichia coli*/100 g of shellfish tissue. Three were put on the market within two days, and the other 102 samples were relocated to basins adjacent to the farms with seawater changed twice a day via tidal events. The other 282 samples were produced in class B areas and underwent depuration prior to analysis.

Ireland, Oysters

In a study undertaken in Ireland to investigate norovirus contamination in oysters, *Crassostrea gigas*, from a shellfishery over the 24-month period from October 2007 to September 2009, 18 oysters were obtained monthly from an area closed due to a previous norovirus outbreak. The harvest area may have been impacted by discharges from wastewater treatment plants, including one located approximately 1 km from the harvest area that provided ultraviolet disinfection in addition to secondary treatment, and another about 10 km from the site that provided secondary treatment only.

Table 1. Norovirus presence in oysters.

Sampling Site	Collected	Negative Samples	Positive Samples
All	387	352	35
Supermarket	82	68	14
Packers	141	127	14
Producers	164	157	7

The data demonstrated the diversity of norovirus genotypes that can be present in sewage-contaminated shellfish.

Total GI and GII norovirus concentrations in norovirus-positive oysters ranged 97-20,080 genome copies/g of digestive tissue and displayed a strong seasonal trend, with greater concentrations during the winter months. The norovirus concentrations were similar during both years of the study.

Although norovirus GII.4 is responsible for the vast majority of outbreak reports, multiple norovirus genotypes were identified during the study: GI.4, GI.3, GII.4, GII.b, GII.2 and GII.e. Norovirus GI.4, the most frequently detected genotype, was present in 99.9% of positive samples. This was followed by GII.4 at 43.7% and GII.b at 37.5% of positive samples.

The data demonstrated the diversity of norovirus genotypes that can be present in sewage-contaminated shellfish. A disproportionate number of non-norovirus GII.4 genotypes can be found in environmental samples compared to the number of recorded human infections associated with non-norovirus GII.4 genotypes.

Portugal, Various Species

Approximately 2,000 bivalve shellfish consisting of Asian clams, *Curbicula fluminea;* native clams, *Ruhitapes decussates*; tellina clams, *Tellina crassa*; surf clams, *Spisula solida*; clock clams, *Dosinia exoleta*; razor clams, *Ensis* species; mussels, *Mytilus* species; flat oysters, *Ostrea edulis*; and cockles, *Cerastoderma edule*; were collected from 10 harvesting areas classified from A to C in the north and center of Portugal from March 2008 and February 2009. The shellfish were grouped into 49 batches based on species and collection sites.

Viral contamination was detected throughout the year in all shellfish species and in all collected areas, independently of their harvesting area classifications. Overall, 67% of all analyzed batches were contaminated by at least one of the studied viruses – norovirus, hepatitis A virus and enterovirus – while the simultaneous presence of two and three viruses was detected in 22% and 6% of the batches, respectively. Of the three viruses, norovirus was detected in 37% of the batches. All strains belonged to genotype GII.4.

Spain, Mussels

A total of 81 mussel samples were obtained over the 18-month period from October 2010 to March 2012 from seven harvesting areas in Rio do Burgo, A Coruna in northwestern Spain. Five of the harvesting areas were classified as B, and two were classified as C.

Noroviruses were detected in 49.4% of the cases, reaching contamination levels from 5.9 x 10^3 to 1.6 x 10^9 copies/g digestive tissue for GI and from 6.1 x 10^3 to 5.4 x 10^6 copies/g digestive tissue for GII. Norovirus strains were assigned to genotypes GI.4, GII.4 and GII.6.

Turkey, Mussels

Mussels were collected along the Bosphorus Coast near Istanbul, Turkey, during 2008-2009. Of the 320 mussel samples collected, five were found to be positive for norovirus genotype GII. No genotype GI was detected. Norovirus genotype GII was present in samples collected in October, November and December 2008, and February and July 2009.

Poland, Mussels

Wild mussel samples were collected from three sites along the Polish coast in the Baltic Sea. In total, 120 shellfish were tested as pooled samples. Norovirus GI was detected in 22 (18.3%) of the samples, and norovirus GII was detected in 28 (23.3%) of the shellfish. Nucleotide sequence analysis of the detected norovirus GII strains showed 97.3 to 99.3% similarity to the GII.4 virus strain.

Belgium, Various Species

Shellfish and fishery products in Belgium were analyzed from October 2012 to March 2013 for the presence of norovirus. For the intact oysters, mussels and clams, 21 of 65 samples from 12 of 34 batches were positive for noroviruses. Nine samples contained quantitative norovirus levels at 3,300-14,300 copies/g shellfish tissue.

For semi-processed scallops and common sole rolls with scallop fragments, 29 of 36 samples from all eight batches were positive for noroviruses. Seventeen samples contained quantitative noroviruses levels at 200-1,800 copies/g tissue. Norovirus genotypes GI and GII were identified in samples, while some samples contained both GI and GII.

U.S. Shrimp Imports From Mexico, India Up With Rise In Shell-On

Imports of shell-on shrimp from Mexico jumped in August.

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Summary:
While Mexico and India shipped more shrimp to the United States this August than a year ago, other major supplying countries were down in volume. Shell-on imports saw a minor rise, with peeled production down a bit. August imports of whole salmon were at their highest annual volume to date. Salmon fillet imports from Chile and Europe reflected the highest total seen in the past four years. Both the fresh and frozen tilapia fillet markets continued to show downward pricing pressure in August. Frozen tilapia imports leveled out over the past two months, but still remained near record highs. Imports of channel catfish increased marginally from July following a seasonal pattern. The catfish market was holding a firm undertone, as *Pangasius* prices fell.

Total shrimp imports to the United States in August were down 10.6% in volume when compared to those of August 2014 (Table 1). However, this year's eight-month total was 3.3% ahead of the January-August 2014 total.

India continued to ship significant quantities of shrimp to the U.S., sending 34.1% more shrimp this August than last year and about 35.0% more year to date (YTD). Mexico shipped some 157.0% more shrimp this August than a year ago. However, all other major supplying countries were down for the month: Indonesia (-13.7%), Ecuador (-20.4%), Vietnam (-40.0%), Thailand (-4.2%) and China (-26.6%).

Shell-on imports, including easy-peel, were up 2.6% for August and 6.3% higher YTD. India continued to ship more shell-on shrimp, and Ecuador shipped less. Indonesia shipped 25.4% less in August, but remained up 21.7% YTD. Mexico increased shipments 92.5% in August.

In August, imports of 26-30 and larger-count shell-on shrimp were all higher, especially 21-25 and larger. Meanwhile, imports of 31-40 and smaller shrimp were down. Mexico shipped significantly more 31-40 and 41-50 shrimp in August.

Peeled imports in August were 4.1% lower, but remained slightly positive YTD. Headless, shell-on (HLSO) and peeled 13-15 and larger Asian white shrimp have been barely steady to weak, with discounting noted. 16-20 and smaller shrimp have turned steady to full steady at listed levels for a fair demand.

Overseas offerings, particularly from India, have firmed as raw material prices have trended higher. Packers there are reported to be actively processing orders for fourth-quarter delivery with limited current selling interest. Other Asian sources are also mostly full steady to firm.

Ecuador's exports to Asia continue to eclipse those to the U.S. and Europe. As Asian and European activity has increased, replacement offerings to the U.S. have firmed. Particularly on 41-50 count and smaller, the market has moved higher. U.S. buying in Ecuador has been challenging, given the higher replacement values. The market has taken on a full steady to firmer undertone.

As of this writing, there was limited availability of Mexican 21-25 and 26-30 HLSO farmed white shrimp. However, Mexican production should soon dominate the markets for those counts. The market is unsettled, but buying interest for new season production is reported increasingly active.

Head-on shrimp have been steady. Mexican new-season wild white shrimp production is under way and centered on under-15 through 21-25 counts. Offshore production has also begun. The market was unsettled. The brown market continued weak and unsettled under selling pressure as carryover inventories were moved ahead of new-season production. Discounting was likely.

Table 1. Snapshot of U.S. shrimp imports, August 2015.

Form	August 2015 (1,000 lb)	July 2015 (1,000 lb)	Change (Month)	August 2014 (1,000 lb)	Change (Year)	YTD 2015 (1,000 lb)	YTD 2014 (1,000 lb)	Change (Year)
Shell-on	39,703	38,685	2.6%	45,771	-13.3%	306,491	288,449	6.3%
Peeled	44,105	45,987	-4.1%	47,091	-6.3%	317,922	316,342	0.5%
Cooked	11,597	10,682	8.6%	14,357	-19.2%	92,181	94,606	-2.6%
Breaded	6,550	7,827	-16.3%	6,834	-4.2%	65,882	58,193	13.2%
Total	101,955	103,181	-1.2%	114,053	-10.6%	782,476	757,590	3.3%

Sources: Urner Barry foreign trade data, U.S. Department of Commerce.

Whole Salmon, Fillets Set U.S. Import Records

YTD whole salmon imports saw extremely large increases in August.

In August, imports of salmon to the U.S. continued the year 11.6% higher year to date (YTD) (Table 2). Total month-to-month data revealed an increase of 6.3% when compared to July. Fresh Atlantic whole fish imports were up 44.6%, while imports of fresh fillets were up 4.5% YTD. And when comparing the August 2015 imports to those for August 2014, we saw a 14.7% increase.

The overall salmon market was about steady in the Canadian and Chilean fillet markets. The European whole fish market was a bit unsettled, with both higher and lower offerings noted.

Whole Fish

YTD whole fish imports continue to see extremely large increases – up 44.6% in August. Canada and, to a smaller extent, Norway are the drivers, with 94.6 and 130.9% increases, respectively. The month-to-month data revealed an increase over July volume of 4.3%. When comparing to August of 2014, imports saw a 27.2% increase this August. With months left in the year, imports of whole salmon are at their highest annual volume to date.

August imports into the Northeast continued to see large jumps compared to 2014. The market in the Northeast has been about steady. Supplies were adequate to fully adequate. Imports into the Northeast were falling below levels seen in 2013. All sizes of Northeast whole fish were trending below their three-year price averages.

Imports of European whole fish during August were slightly above those of last year at the same time. The European whole fish market was somewhat unsettled, with both higher and lower offerings noted. 6-7s took a dive and fell back below the three-year price average.

Imports from Canada on the West Coast continued to see large increases, reaching the highest volume to date. The market in the West, like the Northeast, was about steady. Supplies of smaller whole fish were more readily available than those of larger whole fish. Overall demand was moderate to fair. All sizes listed trended below their three-year averages.

Fillets

U.S. imports of fresh salmon fillets reached 188.7 million lb in August – back to the highest YTD volume on record. Month-to-month data, additionally, showed a 17.4% increase when compared to July figures. Also, when comparing imports to last year at the same time, there was a 22.7% increase.

With 141.6 million lb of U.S. imports thus far this year (up slightly YTD), Chile was the driver of these increases. Norway continued to see double-digit YTD increases up 39.4% through the end of August.

Fillet imports from Chile and Europe totaled 188.7 million lb, the highest total seen in the past four years, but the Chilean fillet market was barely steady to weak. Supplies of fillets out of Chile ranged adequate to fully adequate for a quiet demand. The undertone remained barely steady. All sizes were trending below the three-year price average.

Table 2. Snapshot of U.S. salmon imports, August 2015.

Form	August 2015 (lb)	July 2015 (lb)	Change (Month)	August 2014 (lb)	Change (Year)	YTD 2015 (lb)	YTD 2014 (lb)	Change (Year)
Fresh whole fish	19,595,950	18,796,167	4.26%	15,401,566	27.23%	163,512,204	113,097,011	44.58%
Frozen whole fish	282,750	600,282	-52.90%	674,685	-58.09%	3,413,374	5,157,918	-33.82%
Fresh fillets	26,469,422	22,555,500	17.35%	21,572,869	22.70%	188,744,167	180,575,090	4.52%
Frozen fillets	5,644,880	6,939,629	-18.66%	7,670,005	-26.40%	54,435,139	68,586,773	-20.63%
Total	51,993,002	48,891,578	6.34%	45,319,125	14.73%	410,104,884	367,416,792	11.62%

Sources: Urner Barry foreign trade data, U.S. Department of Commerce.

Whole Tilapia Imports Flat, Ecuador Fillets Recovering From Earlier Declines

August imports of frozen whole tilapia remained flat as the value of the U.S. frozen tilapia supply was at its lowest level in years.

Both the fresh and frozen tilapia fillet markets continued to show downward pricing pressure in August. U.S. imports of frozen tilapia leveled out over the past two months, but still remained at near-record highs.

Whole Fish

August imports of frozen whole tilapia remained flat when compared to those of the previous month and the same month a year ago (Table 3). Still, year-to-date (YTD) volumes were above those seen last year, but only modestly.

Fresh Fillets

Year-to-date imports of fresh fillets were only 1.9% below year-ago levels, but monthly imports since March have fallen under the three-year price average.

Shipments from top supplier Honduras were down nearly 9% compared to this time last year. Imports from Colombia, now the second-largest supplier of this commodity, surpassed those from Costa Rica to total 7.9 million lb YTD – an increase of 39% compared to a year ago.

Former top supplier Ecuador appears to be recovering after years of declining shipments. In 2015, shipments from Ecuador are up nearly 7% compared to 2014 volume. Shipments from Mexico resumed in July after no product was sent in June. August figures revealed a slight increase. Overall, Mexico's shipments to the U.S. were down 20% from those of a year ago.

Market prices adjusted downward during March and remained barely steady ever since. A seasonally soft demand explained the bearish undertone in the market. In addition, replacement costs fell to their lowest level since November 2013 – below the upward support trend seen since June 2012.

Frozen Fillets

August imports of frozen tilapia fillets increased 16% from the previous month and 14% from the same month a year ago. YTD figures are currently at the second-highest import volume ever.

The current situation in the tilapia market is a bit tricky. High prices from the fourth quarter of 2013 through the middle of last year pushed farmers in China to ramp up production. Seeding was strong going into the second half of 2014, including for commitments made in the first quarter of 2015.

Increased production mixed with lackluster demand – largely the result of record-high prices in 2014 – forced replacement costs and prices in the U.S. to drop. This was spurred by buying resistance from U.S. importers at previously high levels. Many U.S. importers reported heavy high-priced inventories going into the final three months of 2014. However, there have been reports of many plants in China operating under capacity, mainly due to a shortage of raw materials.

In April, it was reported that seeding this year could be 30% lower than last year, mostly due to lower prices. Recent reports allegedly claimed hatchery sales were off around 30% compared to last year. This strained the market, as many plants in China have been buying scarce raw materials at rising prices.

However, shipments arriving in the U.S. since June posted low replacement costs not seen in years. In fact, August figures showed replacement costs falling to their lowest level since October 2010. This meant the value of the U.S. frozen tilapia supply was at its lowest level in years.

Finally, many importers reported that replacement offerings for late 2015 arrivals into the first quarter of 2016 were at significantly higher levels than what they have been paying over the last 10 months or so. So the situation is blurry at best. Costs of currently held inventories are at multiyear lows, but quotes for purchase orders for late 2015 and early 2016 deliveries are significantly higher.

Table 3. Snapshot of U.S. tilapia imports, August 2015.

Form	August 2015 (lb)	July 2015 (lb)	Change (Month)	August 2014 (lb)	Change (Year)	YTD 2015 (lb)	YTD 2014 (lb)	Change (Year)
Fresh fillets	4,471,010	4,422,612	1.09%	4,843,395	-7.69%	38,535,627	39,284,376	-1.91%
Frozen whole fish	8,200,818	8,146,689	0.66%	8,088,869	1.38%	59,692,355	53,289,897	12.01%
Frozen fillets	30,050,698	25,863,122	16.19%	26,374,996	13.94%	227,760,344	217,565,515	4.69%
Total	42,722,526	38,432,423	11.16%	39,307,260	8.69%	325,988,326	310,139,788	5.11%

Sources: Urner Barry foreign trade data, U.S. Department of Commerce.

U.S. *Pangasius*, Catfish Imports Steady

The *Pangasius* market remained barely steady in August, with year-to-date (YTD) import volumes to the United States at record highs. The catfish market was steady at listed levels, with many reporting supply shortages in the U.S.

Channel Catfish

Imports of channel catfish in August increased marginally from the previous month following their suggested historical seasonal pattern (Table 4). On a year-to-date basis, imports were down 19% from 2014 and 2013 levels. Therefore, it made sense that prices adjusted slightly higher, all else equal. Replacement costs in July reached their highest level since March 2012, when replacement reached U.S. $3.64/lb, but then adjusted downward in August. Still, the market in the U.S. was holding a firm undertone, with many reporting supply shortages.

Pangasius

Pangasius imports in August declined when compared to those of the previous month and the same month a year ago, and against the three-year average. Imports in 2015 have not followed a seasonal pattern, but have remained strong – averaging nearly 20 million lb/month. Imports through August remained at a record high 156.4 million lb, an 18.0% increase over a year ago and up 3.3% since 2013.

Note that European data through June showed how shipments to the U.S. market have surpassed those in Europe for the third straight month. This placed year-to-date imports to the U.S. up 2.3% over shipments to Europe.

Prices in the U.S. market retreated in June and then fell again to record lows by late August. The general undertone in the U.S. was soft, with traders reporting heavy inventories. In addition, August data showed replacement costs fell to levels not seen since September 2006.

Table 4. Snapshot of U.S. catfish imports, August 2015.

Form	August 2015 (lb)	July 2015 (lb)	Change (Month)	August 2014 (lb)	Change (Year)	YTD 2015 (lb)	YTD 2014 (lb)	Change (Year)
Pangasius	16,971,394	19,979,423	-15.06%	18,894,692	-10.18%	156,361,625	132,316,749	18.17%
Channel catfish	191,079	38,999	389.96%	265,174	-27.94%	7,518,852	9,257,347	-18.78%
Total	17,162,473	20,018,422	-14.27%	19,159,866	-10.42%	163,880,477	141,574,096	15.76%

Sources: Urner Barry foreign trade data, U.S. Department of Commerce.

Double-Stranded RNA Against WSSV Genes Provides Antiviral Protection In Shrimp

At the end of the experiments, most of the animals treated with dsRNA against WSSV genes survived and had low rates of WSSV infection.

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Summary:
Silencing genes in white spot syndrome virus (WSSV) with critical roles in replication could provide a strong antiviral effect and thus reduce shrimp mortality. The authors therefore established a study to evaluate the antiviral efficacy of double-stranded (ds) RNA against non-structural WSSV genes. A single dsRNA dose of 4 µg/shrimp was enough to trigger an antiviral response against a lethal WSSV challenge. Sequence-specific dsRNA against WSSV genes vp28, vp26 and orf89 effectively inhibited virus replication and reduced mortality in treated shrimp.

The white spot syndrome virus (WSSV) has caused major economic losses to shrimp farmers worldwide since the 1990s. Various field treatments and strategies for controlling WSSV have been tested, showing different degrees of efficacy. Some of these include immunostimulants, administration of recombinant viral proteins, manipulating water temperature, DNA vaccines and RNA interference (RNAi).

WSSV has up to 531 putative genes, some of which may be essential for WSSV infection/replication. Most of the WSSV genes have unknown roles in virus infection. Several studies have used RNAi against WSSV genes, encoding structural proteins involved in virion architecture and virus entry. Other studies have assessed the antiviral efficacy of non-structural WSSV proteins.

It is possible the antiviral efficacy of RNAi molecules depends on the targeted genes. Silencing WSSV genes with critical roles in virus replication might show a stronger antiviral effect and thus reduce shrimp mortality.

Study Setup

The authors therefore established a study to evaluate the antiviral efficacy of double-stranded (ds)RNA against non-structural WSSV genes orf89 and wsv191 in Pacific white shrimp, *Litopenaeus vannamei*, and compare it to dsRNA against WSSV genes vp28 and vp26 under experimental conditions.

Juvenile shrimp from a hatchery in Sonora, Mexico, were tested WSSV-negative by polymerase chain reaction (PCR) analyses. The animals were maintained in artificial seawater with a salinity of 25 g/L, temperature of 27 ± 2° C and continuous areation. Fifty percent water exchange was done every third day to maintain good water quality.

A WSSV inoculum prepared from naturally infected shrimp from Sinaloa, Mexico, was titrated in vivo. For each tenfold serial dilution (10^{-2}-10^{-7}), five shrimp were intramuscularly inoculated and individually kept in 12-L tanks with artificial seawater.

The shrimp were monitored twice daily for clinical signs of WSSV infection and mortality for 10 days. Infectivity and lethal titers were determined to be $10^{5.6}$ SID_{50}/mL and $10^{5.6}$ L.D.$_{50}$/mL, respectively. SID_{50} is the shrimp infectious dose that will result in 50% infected shrimp. L.D.$_{50}$ is the single dose that will cause death in 50% of a group of test animals.

RNAi against four WSSV genes – vp26, vp28, vp191 and orf89 – was produced as dsRNA using a commercial transcription kit following the manufacturer instructions. Table 1 includes information on the specific primers and standard PCR conditions used to amplify the genes.

Table 1. Sequence and expected amplicon size of different PCR primer pairs.

Primer Name	Sequence 5' ⟶ 3'	PCR Product Size (bp)
orf89F1 orf89R1	AGGACCCGATCGCTTACTTTGA CAAGAAACCGGGAGGGATTTTC	483
wsv191F1 wsv191R1	AAGTGGGTGCGCAACAAAATA TGTAGAGGGCATGAGGGATAG	451
vp28F3 vp28R3	ATGGATCTTTCTTTCAC TTACTCGGTCTCAGTGC	615
vp26F4 vp26R4	ATCCAACCAACACGTAAAGG GGAAAATCTACATCTGTTGTGC	580
LacZF2 LacZR2	ACCAGAAGCGGTGCCGGAAA CCACAGCGGATGGTTCGGAT	1,012
β-ActinF2 β-ActinR2	GAAGTAGCCGCCCTGGTTG CGGTTAGCCTTGGGGTTGAG	416
BLOCK-iT. T7	GATGACTCGTAATACGACTCACTA	

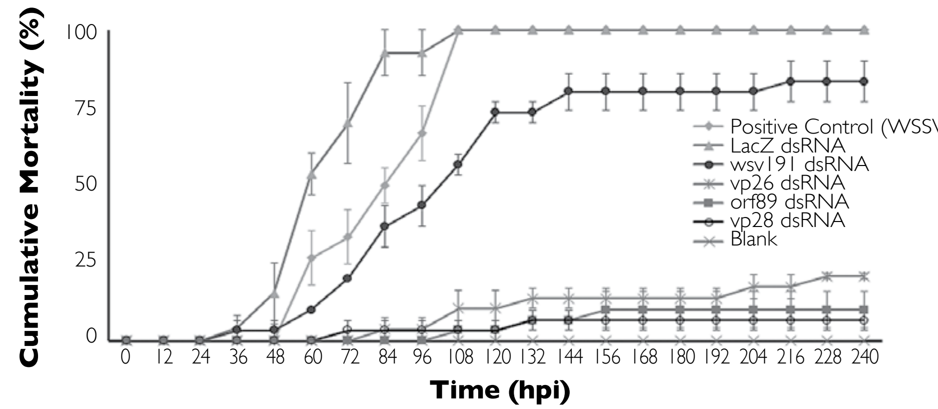

Figure 1. Mean cumulative mortality of shrimp treated with dsRNA against WSSV genes. Groups of mock-treated shrimp, LacZ dsRNA and a blank group not challenged with WSSV nor treated with dsRNA are included.

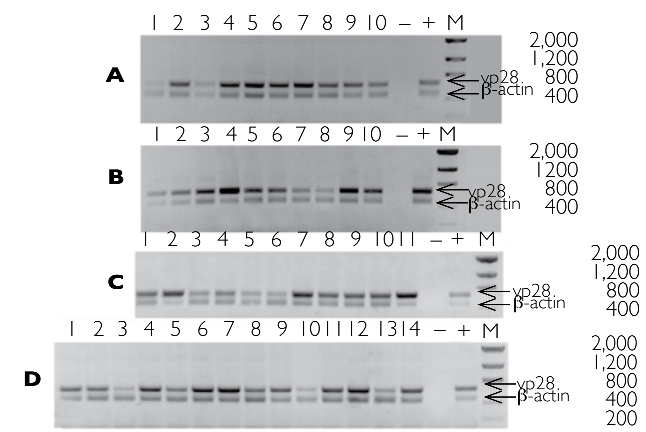

Figure 2. PCR analyses of moribund and dead shrimp in (A) mock-treated; (B) LacZ dsRNA; (C) WSSV-positive shrimp treated with dsRNA against vp28 (lanes 1-2), orf89 (lanes 3-5) and vp26 (lanes 6-11); (D) WSSV-positive shrimp treated with wsv191 dsRNA. β-actin indicates an internal control, along with a negative control (–), WSSV positive control (+) and molecular weight marker (M) size in base pairs.

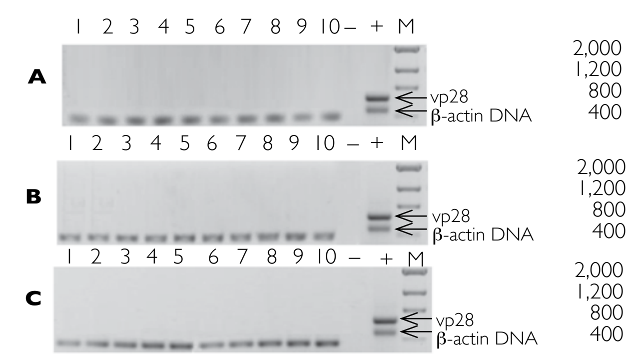

Figure 3. RT-PCR from surviving shrimp treated with dsRNA against (A) vp28, (B) orf89 and (C) vp26. No vp28 cDNA amplification was observed. β-actin indicates an internal control, along with a negative control (–), WSSV positive control (+) and molecular weight marker (M) size in base pairs.

Treatments

Groups of 10 WSSV-free *L. vannamei* were acclimatized for 24 hours in 80-L tanks containing artificial seawater at 25-g/L salinity, a temperature of 25 ± 2° C and with continuous aeration. Each group was respectively inoculated via 4-µg injections with one of the dsRNAs.

A group was treated with 40 µL dsRNA against bacterial LacZ gene and used as a control. Another group of 10 shrimp was mock treated with 40 µL phosphate-buffered saline and used as a positive control. Three replicates were done per treatment.

After 48 hours, all the treated animals were intramuscularly challenged with a high dose (2,500 SID_{50} in 50 µL) of WSSV. The shrimp were monitored twice daily for 10 days after the challenge for clinical signs of disease and mortality. Moribund and dead shrimp were recorded and removed from the tanks, with tissue samples taken for WSSV detection.

At the end of the experiment, surviving shrimp were sacrificed, and their gills were used for RNA extraction and WSSV PCR analyses. The antiviral efficacy of the different dsRNAs was assessed using cumulative mortality and number of WSSV-infected shrimp.

Results

The mock-treated group and shrimp treated with LacZ dsRNA first showed clinical signs of WSSV infection, such as reduced feeding, erratic swimming and/or lethargy at 24 hours post inoculation (hpi). The first mortalities were recorded at 36 hpi. These two groups had 100% mortality at 108 hpi (Figure 1), and all animals in these groups were WSSV-positive by PCR (Figure 2).

Shrimp treated with vp28 dsRNA showed first mortalities at 72 hpi and final cumulative mortality of 7%. Animals treated with orf89 dsRNA displayed first mortalities at 108 hpi, and their cumulative mortality at the end of the experiments was 10%. Shrimp treated with vp26 dsRNA showed first mortalities at 84 hpi and cumulative mortality of 21%. Animals treated with wsv191 dsRNA showed first mortalities at 24 hpi and final cumulative mortality of 83% (Figure 1).

All dead animals in these treatments were WSSV-positive by PCR. The surviving shrimp did not display clinical signs of WSSV infection and were WSSV-negative by RT-PCR assays (Figure 3).

(Continued on page 65.)

From Bench To Farm

Innovative On-Site Diagnostic Kit Identifies WSSV In Shrimp

In field testing, farm staff readily learned how to use the WSSV detection kits.

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Summary:
Loop mediated isothermal amplification (LAMP) is a highly specific technique that can amplify DNA in isothermal conditions. LAMP has potential applications for clinical diagnosis and surveillance of infectious diseases without requiring sophisticated equipment or skilled personnel. A LAMP-based on-site diagnostic kit for the detection of white spot syndrome virus in shrimp was developed in the Philippines. The kit can amplify the target gene of the pathogen using a single temperature and give results in one hour. Pilot testing provided encouraging results with no false positives.

Global aquaculture production has increased steadily, now producing 90 mmt valued at U.S. $144 billion annually and accounting for 42% of the total fisheries production, according to the latest data from the Food and Agriculture Organization of the United Nations. With the advance of its production and marketability, aquaculture has become an essential source of food and livelihoods worldwide, especially in developing countries.

Research and development – particularly in the areas of disease management and good aquaculture practices – play a major role in increasing production. Disease outbreaks significantly diminish aquaculture production, especially for medium- and small-scale farmers.

The development of novel ways of biosurveillance will significantly affect the industry's sustainability. Early detection of target pathogens, for example, can help mitigate the spread of diseases and prevent massive mortalities.

Many new technologies, however, are still out of reach for many farms due to their high prices and the need for a skilled technical staff to implement molecular diagnosis. Most of the available technology is produced by developed countries and tends to be either unavailable to the local market or simply too expensive and highly technical to be utilized by farm personnel.

LAMP Technology

Loop mediated isothermal amplification (LAMP) is a DNA amplification technique developed by Tsugunori Notomi and his colleagues from Japan in 2000. The highly specific and rapid amplification technique can amplify DNA in isothermal conditions. Unlike polymerase chain reaction (PCR) methods, which amplify DNA by constantly changing temperatures, the LAMP assay uses a single temperature setting during the reaction.

It uses four sets of primers that target six regions of the DNA. It requires a special kind of DNA polymerase, *Bst*, which has strand displacement activity. The amplification procedure creates stem-loop configurations of the target DNA.

After its introduction, LAMP was used as a diagnostic tool and "point of care" system for different human diseases, as well as in detecting pathogens in aquaculture. LAMP has potential applications for clinical diagnosis, as well as surveillance of infectious diseases in developing countries without requiring sophisticated equipment or skilled personnel. Despite its wide use as a diagnostic tool in the laboratory, its application at the farm level is limited, because the preparation of quality DNA templates is still highly technical, and the cost is high.

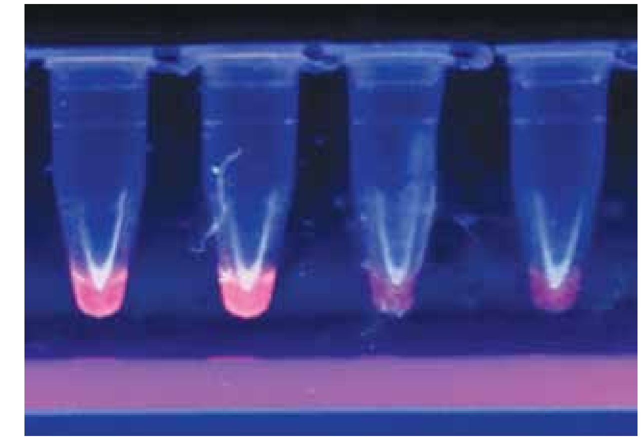
On-Site WSSV Diagnosis

A LAMP-based on-site diagnostic kit, a platform for the rapid detection of white spot syndrome virus (WSSV) designed for the shrimp industry in the Philippines, was developed by a team of researchers from the University of Santo Tomas in Manila, Philippines. The detection tool can amplify the target gene of the pathogen using a single temperature and give results in one hour.

The kit is designed to replace the traditional practice of relying on on-site morphological and physiological observations to determine the occurrence of disease outbreaks. The diagnostic kit includes a locally fabricated heat block that replaces the expensive commercially available equipment. This heat block uses low-cost raw materials in making the machine, which delivers the right balance of effectiveness and simplicity needed in the farm setting. It also includes a sample preparation kit for the LAMP reaction that can extract DNA from the target organ in less than 20 minutes.

The kit is designed to replace the traditional practice of relying on on-site morphological and physiological observations to determine the occurrence of disease outbreaks.

The reagents used in the kit are all inexpensive and readily available. The kit also uses simple dye that can be seen under black light to interpret the assay results. This overall approach eliminates the expensive equipment, tedious sample preparation and visualization of results associated with conventional PCR assays.



Results are viewed using a hand-held black light. The bright tubes show positive reactions.

Field Trials

The diagnostic kit was conceived as a farm-based detection platform. During its pilot testing at selected sites in the Philippines, it provided encouraging results with no false positive results.

The DNA isolation part of the kit provided quality DNA that was also validated in the laboratory. The kit also proved more sensitive than PCR in the farm setting. Using the prototype kit, 87% of the samples tested positive, while PCR testing identified only 25% of the same samples.

The farm personnel performed the procedure with the help of a simple manual and minimal training. They were able to extract the DNA, perform the assay and view results using a hand-held black light.

Perspectives

WSSV occurrence remains a problem for the shrimp industry that causes huge economic losses. As efforts to raise production levels continue, and research interest in aquaculture grows, expansion of the use of biosurveillance methods is also expected.

Beyond the initial testing sytem for WSSV, additional primers for other pathogens in shrimp and fish can be designed using the current diagnostic platform. The technology can then be used by research facilities, universities, government agencies and shrimp farms to become a staple for the detection of WSSV and other infectious diseases in aquaculture in the Philippines and other countries.

Double-Stranded RNA *(Continued from page 63.)*

Treated shrimp were monitored in these experimental units.

Antiviral Response

A single dsRNA dose of 4 µg/shrimp was enough to trigger an antiviral response against a lethal WSSV challenge. Sequence-specific dsRNA effectively inhibited virus replication and reduced mortality in treated shrimp. At the end of the experiments, most of the animals treated with dsRNA against WSSV genes survived and had low rates of WSSV infection. In contrast, shrimp treated with sequence-independent dsRNA (bacterial LacZ gene) became infected and only underwent a delay in time to mortality.

Shrimp treated with dsRNA against vp28 and vp26 were protected against a highly lethal WSSV challenge. Genes vp28 and vp26 encode structural proteins VP28 and VP26, which have been shown to play important roles in virion structure, virus attachment and infection.

The two dsRNAs against the non-structural WSSV genes orf89 and wsv191 showed that silencing the orf89 gene was effective in reducing virus replication and shrimp mortality in a way similar to that observed with vp28 dsRNA. In contrast, dsRNA against wsv191 had the least antiviral effect of the four sequence-specific dsRNAs tested.

Perspectives

The functions of the two non-structural proteins encoded by these WSSV genes may help explain the differences in antiviral activity. Gene orf89 is expressed early during virus replication and encodes a viral protein that has a negative regulatory function. This gene may have an essential early role during virus infection/replication, and therefore its silencing greatly affected the outcome of WSSV infection. In contrast, gene wsv191 probably is not an essential gene for WSSV replication, despite the fact it may encode a non-specific endonuclease with both DNAse and RNAse activities. Its silencing was not significant in inhibiting virus replication.

Limited Decomposition Enhances PCR Detection Of AHPND *Vibrio* In Shrimp

Juvenile shrimp were preserved in ethanol before dissection and further processing in the laboratory.

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Summary:
A study confirmed the utility for improved polymerase chain reaction (PCR) detection of the *Vibrio* bacteria that cause acute hepatopancreatic necrosis disease (AHPND) in asymptomatic shrimp by permitting the shrimp to expire and decompose for several hours prior to preservation and PCR processing. If shrimp to be PCR tested for AHPND *Vibrio* are not sick when collected, the authors recommend bacteriological culture enrichment of their anterior digestive tract tissues or allowing the shrimp to decompose to increase the density of the targeted bacteria to improve detection sensitivity.

Reports have been issued over the past several years regarding the presence of acute hepatopancreatic necrosis disease (AHPND) in various countries. Today we know the AHPND-causing strains of *Vibrio parahaemolyticus* can occur in the anterior digestive tracks of shrimp at a cell density below the detection limit for polymerase chain reaction (PCR) tests without causing disease. For PCR detection of these subclinical carriers, modification of how shrimp are processed following collection from ponds or tanks make a difference to the detection sensitivity of the PCR test process.

PCR Primers

Molecular testing by PCR has been employed since 2013 for the detection of AHPND *V. parahaemolyticus*. Over the past two years, improved PCR primers that specifically target DNA sequences in the genes that code for the Pir toxins were developed in the laboratories of Dr. Timothy Flegel in Thailand and Dr. Donald Lightner in Arizona, USA. Additionally, commercial PCR detection kits are readily available for AHPND diagnosis.

A study by the authors examined the utility for improved PCR detection of AHPND-competent *V. parahaemolyticus* in asymptomatic and otherwise healthy-appearing shrimp from ponds where disease was not apparent by permitting sampled shrimp to expire and decompose for several hours prior to ethanol preservation.

Study Parameters

The study applied three PCR primers. Primer set Vpldh reacts with DNA sequences in a gene marker for lecithin-dependent hemolysin, which is specific for *V. parahaemolyticus*. Primer set AP4 is a nested PCR detection method with greater sensitivity that was updated from Flegel's earlier AP3 test. AP3 detects the *Pir*-like *ToxA* and *ToxB* genes, as does the primer set VpPirAB reported by Han and co-authors. These genes code for the protein toxins that are the direct cause of AHPND in shrimp.

Samples of shrimp were randomly collected by cast netting in four ponds on a farm in Latin America where intermittent mortality of shrimp had been previously observed. Shrimp mortality was not observed at the time the samples were collected.

For each pond, one batch of shrimp was collected and identified as O, P, Q and R. From each batch, two groups of shrimp were sampled and tested. Group 1 (O1, P1, Q1, and R1) consisted of a larger number of individual shrimp sacrificed quickly by injection, followed by immersion in 95% ethanol.

Group 2 shrimp (O2, P2, Q2, and R2) were gathered and placed outside the ponds, where they were allowed to expire. The Group 2 shrimp where held at room temperature to decompose for four to five hours prior to preservation of individual shrimp by injection and immersion with 95% ethanol.

Two of the ponds sampled contained Pacific white shrimp, *Litopenaeus vannamei*, and two held black tiger shrimp, *Penaeus monodon*. The four ponds were stocked at different times, so the shrimp sizes varied among the ponds, although all of the shrimp were juveniles. The numbers of individual shrimp are listed by species and processing method in Table 1.

Table 1. Breakdown of preserved shrimp samples.

Species	Preserved Immediately	Preserved After Decomposition
Litopenaeus vannamei	33	16
Penaeus mondon	51	16
Total	84	32

Laboratory Processing

At the PCR laboratory, each shrimp was processed as an individual sample. For the smaller shrimp (Groups O1-2, P1-2, Q1-2), the heads were severed from the tails, and the eyes were removed, leaving the main portions of the heads. The heads were processed further for PCR testing.

The shrimp of larger size (Groups R1-2), were individually dissected. Their stomachs and hepatopancreases were removed and processed further for PCR testing as individual samples. The DNA extracted from each shrimp was assayed with the three primer sets.

Results

As shown in Table 2, the shrimp in Group 1 (O1, P1, Q1 and R1) had been fixed live. The PCR results showed between 3.3% and 16.7% were positive by Vpldh, and none were positive by VpPirAB and AP4.

For Group 2 (O2, P2, Q2, and R2), in which shrimp were allowed to decompose before fixation, between 25% and 100% of the individual shrimp sampled positive by Vpldh. However, zero, 12.5% and 100% were positive by VpPirAB, and zero, 37.5% or 100% were positive using AP4 primers.

Interpretation

The PCR test data demonstrated a number of things. First, the Vpldh primer results indicated that non-AHPND *V. parahaemolyticus* was common flora in the pond environment on the farm because it was present in some of the shrimp sampled from the four ponds.

Second, in Group 1, no shrimp tested PCR positive by the VpPirAB or AP4 primer sets in the samples from the four ponds. This outcome demonstrated that shrimp sampled and sacrificed shortly after collection gave PCR test results that would be compatible with an interpretation that AHPND-competent *V. parahaemolyticus* was either not present in the shrimp sampled or was below the detection level for the PCR primers applied.

Third, the decomposition of Group 2 shrimp prior to ethanol preservation provided a means to increase the prevalence of shrimp that were PCR positive for the three primer sets. Moreover, decomposition improved test sensitivity for the AHPND-competent *V. parahaemolyticus*, as two of the four pond samples tested positive.

Fourth, in this study, the findings indicated the AP4 primer set was a bit more sensitive than the VpPirAB set for detection of AHPND-competent *V. parahaemolyticus.*

The simplest explanation for improved PCR test results was that decomposition provided the nutrient substrate to support bacterial multiplication. This resulted in increased biomass of the bacteria in the shrimp carcasses, which provided sufficient DNA for the PCR primers to react and provide a positive test outcome.

Small containers were used to hold shrimp for decomposition after collection.

Recommendations

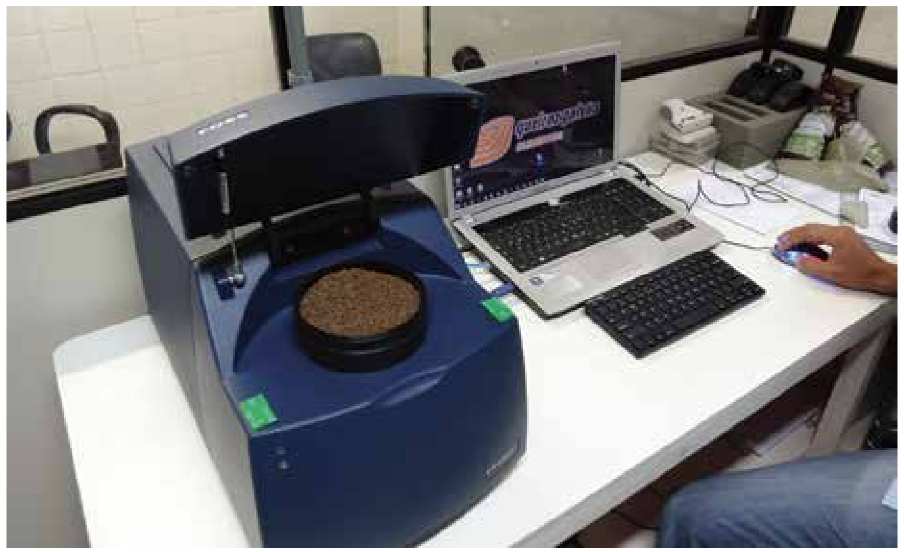
PCR testing is a viable method for distinguishing between pathogenic APHND-competent and benign strains of *V. parahaemolyticus*. For circumstances in which asymptomatic shrimp will be sampled and tested by PCR for the pathogenic *V. parahaemolyticus*, the authors recommend the removal of the stomach and hepatopancreas from each shrimp and their inoculation in suitable bacteriological media for five to 12 hours prior to sample preservation in ethanol. Optionally, following collection from the pond, shrimp should be set aside to decompose for at least five hours before the individual shrimp are injected with ethanol for fixation. These preliminary steps prior to ethanol fixation will markedly improve the sensitivity of PCR detection of AHPND-competent *V. parahaemolyticus.*

Table 2. Test results of study.

					PCR Test Positives		
Group	Species	Sample Count	Status at Fixation*	Weight (g)	Vpldh	AP4	VpPirAB
O1	*L. vannamei*	18	Live	0.3	16.7%	0	0
O2	*L. vannamei*	8	Dead	0.3	100.0%	37.5%	12.5%
P1	*P. monodon*	21	Live	1.5	14.3%	0	0
P2	*P. monodon*	8	Dead	1.5	100.0%	100.0%	100.0%
Q1	*P. monodon*	30	Live	2.0	16.7%	0	0
Q2	*P. monodon*	8	Dead	2.0	87.5%	0	0
R1	*L. vannamei*	15	Live	4.5	3.3%	0	0
R2	*L. vannamei*	8	Dead	4.5	25.0%	0	0

* Dead shrimp were dead for four to five hours prior to ethanol fixation.

NIRS Technology Ensures Shrimp Feed Quality At Farm Level



NIRS can perform multiple analyses within minutes. The NIRS concept is based on prediction models using calibration curves for desired nutrients.

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Summary:
Feed plants deal with varied raw materials, formulations and feeds for different animals, which can lead to quality issues and nutrient variations. When assessing feed quality at shrimp farms, traditional wet chemistry methods take considerable time and physical space, as well as qualified personnel to handle the specialized equipment. However, near-infrared spectroscopy (NIRS), a quick evaluation technology that compares the contents of samples to calibration curves for different ingredients, has been successfully applied in Brazil to ensure feed quality standards at the farm level.

The nutrient quality and biological performance of shrimp feeds result from the nature and quality of the raw materials used in their composition, as well as the formulation tools, equipment and manufacturing methods used in their production.

Feed plants ensure the quality of their feed by adhering to strict quality controls. This starts with the purchasing department, which works with suppliers to set quality standards for raw materials. Quality adherence continues through controls that certify the physical and chemical qualities of finished feeds. However, on a daily basis, feed plants usually deal with several types of raw materials, formulations and feeds for different animals, which can lead to quality issues and nutrient variations not easily detectable at the manufacturer level, let alone by farmers.

Near-Infrared Spectroscopy

From the farmer's perspective, little can be done to deal with these possible variations in feed quality other than making the best selection of feed suppliers while continuously evaluating farm results. However, near-infrared spectroscopy (NIRS), a new quick evaluation technology, has been successfully applied in Brazil to ensure feed quality standards at the farm level.

Potiporã Aquacultura, the largest shrimp farm in Brazil, uses NIRS to evaluate the feed it acquires from third parties. Located in Rio Grande do Norte, Brazil, Potiporã is part of the vertically integrated maturation, hatchery, growout and processing operations owned by Queiroz Galvão Alimentos S.A. The 960-ha farm used 4-ha growout ponds to produce 7,000 mt of Pacific white shrimp, *Litopenaeus vannamei*, in 2014.

Wet Chemistry Versus NIRS

When choosing a method to assess feed quality at a shrimp farm, one should address its accuracy, speed, cost, repeatability and ease of operation. Traditional wet chemistry methods meet some of these criteria. However, they often take considerable time and require physical space, as well as qualified personnel to handle the specialized equipment and reagents. Such conditions make this method impractical for the quick decision making often required by large-scale commercial shrimp operations.

NIRS can perform multiple analyses within minutes. The NIRS concept is based on prediction models using calibration curves for desired nutrients. The curves are based on a database previously developed from chemical analyses of a wide and diversified universe of samples. Feed is analyzed by collecting a ground or unground sample of about 100 g, followed by its exposure to the equipment, which provides results within seconds.

Calibration curves are usually designed to estimate moisture, crude protein, fat, crude fiber, ash, calcium and phosphorus values for selected raw materials. At Potiporã farm, calibration curves were initially standardized with 460 feed samples individually analyzed by wet chemistry.

In addition to the investment involved in the acquisition of NIRS equipment, there are also costs to develop these calibration curves. This effectively limits the application of NIRS to large-scale aquaculture operations that rely on third-party feed suppliers.

Feed Quality Standards, Protocol for Analyses

Potiporã uses one starter feed with two particle sizes and two types of growout feed, one for semi-intensive ponds stocked at 30 shrimp/m^2 without mechanical aeration and another for intensive conditions with mechanical aeration and up to 100 shrimp/m^2. Therefore, quality standards were set for each feed type.

In Brazil, feed bags carry a tag indicating the minimum and maximum levels of basic nutrients. Originally, Potiporã adopted these quality standards set by the feed manufacturer, allowing a variation of ± 5.5%.

To establish levels within ranges that would not compromise the nutrient balance of the diets, feed quality standards were revised after 800 readings performed by NIRS. In this case, a narrower variation of ± 3.0% was set for each parameter (Table 1).

All feed that enters the farm is analyzed before truck unloading and storage. If the product does not meet the established quality standards, feed is rejected, and the supplier is informed. If feed falls into desirable standards, it is unloaded.

Perspectives

Driven by availability due to shifts in prices and/or seasonality, seemingly simple changes of ingredients or their inclusion levels in a feed formula can lead to significant changes in key dietary nutrients. Set minimum quality standards and nutrient compositions for raw materials should be analyzed upon arrival of feed at the plant to allow adjustments in formulations before feed manufacturing.

Aerial view of shrimp growout ponds at Potiporã Aquacultura. It is the largest shrimp farm in Brazil.

However, continuous sampling and analysis of all raw materials may not always be feasible, and formulators often need to rely on expected values from a database. This can negatively affect the nutrient value of a feed and limit the ability to modify the amount and bioavailability of critical nutrients such as protein and amino acids.

The unique feed quality assessment program developed by Potiporã has safeguarded the enterprise from potential financial losses associated with undesirable feed quality. The continued surveillance through NIRS has reduced the uncertainties and variables that affect shrimp farming.

With a comprehensive database on hand, the company has established a feed purchasing policy aligned with farm needs for nutritious feed products. Calibration curves are now under development to allow NIRS feed analysis for essential amino acids and fatty acids.

Table 1. Chemical quality standards for shrimp feeds used by Potiporã Aquacultura. (Variation = ± 3.0%).

	Nutrient Composition (% of diet, as-is basis)		
Parameter	Starter Diet	Semi-Intensive Diet	Intensive Diet
Crude protein (minimum)	40.0	35.0	38.0
Digestibility (minimum)*	80.0	80.0	80.0
Fat (minimum)	10.0-11.0	9.0-10.0	10.0-11.0
Total fiber (maximum)	2.0-3.0	2.0-3.0	2.0-3.0
Ash (maximum)	1.3	1.2	1.3
Calcium (minimum – maximum)	4.0	4.0	4.0
Total phosphorus (minimum)	12.0	11.0	12.0
Moisture (maximum)	8.0-12.0	8.0-12.0	8.0-12.0

* Digestibility in pepsin at 0.0002%.

Novel Air-Based System Transfers Large Salmon During Harvest

In testing, sliding the fish by hand into the outlet channel allowed manual orientation of the salmon to test fish transfer through the system.

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Summary:
To evaluate the application of an air pressure-based transport method within a recirculating aquaculture system, the authors performed testing with harvest-size salmon at The Conservation Fund Freshwater Institute. The system uses air to gently pull and then push fish through a flexible hygienic tube with minimal water. The system tube was installed quickly using a wire cable to suspend the tube. The salmon were transferred through the tube from the accelerator pump to the finishing/purging tanks in only a few seconds and with minimal impacts to the fish.

Various systems have been utilized to move culture animals between production units or into transport vehicles at harvest. Harvest “pumps,” for example, transfer fish from ponds or tanks along chutes or through tubes along with an artificial flow of water generated by the pumps.

A new transfer system based on compressed air moves fish from unit to unit through flexible tubing. The air pressure differential introduced across the fish results in a net force that propels the fish through the tube. The patented new Whooshh™ system uses very little water (less than 34 L/hour), which is only used to lubricate the interior of the tube.

Technology Applications

The technology was originally developed to safely and gently transport delicate food objects such as apples, citrus and peaches. It was later tested to move salmon over dams instead of using fish ladders, trap and haul, or other fish-handling equipment.

For the past two years, the Washington Department of Fish and Wildlife has used the transport system to separate endangered Chinook salmon from hatchery-bound fish by moving the hatchery-bound fish up a steep river bank into waiting hatchery trucks. Previously, hatchery workers had to dump the fish into totes and hand carry them up the bank to the tanker truck.

This past summer, the volitional entry function of the system was tested at the Buckley Diversion Dam on the White River in Washington, USA. The test demonstrated that fish will follow the water flow and swim into the system on their own. In addition, the air-based system is being used to move harvested fish, and is in use in Norwegian salmon-processing plants.

RAS Test

To evaluate the application of the transport method within a recirculating aquaculture system, the authors performed testing at The Conservation Fund Freshwater Institute in Shepherdstown, West Virginia, USA. Atlantic salmon of about 4-kg weight were harvested from the facility’s 150-m^3 growout tank using a clam-shell grader to crowd fish to the sidewall drain box, which typically receives 90-95% of the flow through the tank and is relatively free of settleable solids. To harvest the fish, a normally vertical perforated door through the sidewall drain was lowered to open.

When the harvest wound down to only a few dozen remaining fish in the tank, the crowded fish were netted by hand and directed into the sidewall drain box without lifting them out of the water. A dewatering rack installed in the drain box separated the water flow from the fish before the fish were slid by hand into the box and an outlet sluice channel. Sliding the fish by hand into the outlet channel allowed manual orientation of the salmon either head first or tail first to test fish transfer through the system.

In the test, the outlet sluice channel outlet was connected to the intake of the system pump house or “accelerator.” The system uses air to first gently pull and then push the salmon through a flexible hygienic tube with minimal water to substantially increase the distance, height and speed that large harvest-size salmon can be transferred.

The system tube was installed quickly using a wire cable to suspend the tube. The salmon were then transferred through the tube from the accelerator to the finishing/purging tanks in only a few seconds. Unlike gravity-feed fish transfer pipes, this system allows the fish to move up and over equipment, taking advantage of the unused space above the floor and equipment.

The "accelerator" is attached to the side wall drop box of a tank (left rear). Fish that enter the system are "whooshed" along via the exit at the lower right. The red tube pulls air out of the system. A second tube underneath (not shown) pushes air.

Fish-Friendly

The salmon were oriented with roughly half transferred head first into the system and the other half transferred tail first. To determine if transferring the fish either head first or tail first had any negative impact, the treatment groups were separated and held for six days in the purge/finishing tank.

The results were encouraging. While one salmon was lost immediately after transfer because the outlet of the tube was initially misaligned, and the fish collided with the wall of the tank, the tube was adjusted, and the test continued. Other than that first fish, no dead fish were found in the tank during the six-day depuration/finishing period.

After six days, all of the fish were anesthetized and examined for wounds and bruising. There was little or no bruising on the salmon moved head first. However, some bruising was observed near the tails of a few fish that were transferred tail first.

Based on the bruising pattern, this bruising may have been inadvertently caused when pushing the fish backward into the dewatering PVC pipe used between the sidewall drain box and the entry to the accelerator. An easy modification to this PVC pipe would avoid this problem.

Water Savings

Overall, this system was highly effective in rapidly moving large salmon through a 12-m-long tube. Moving large fish without water offers a huge advantage, particularly in recirculating systems, because typical 30-cm-diameter fish pumps can require a flow of more than 4,000 L/minute to move fish.

Pumping these flows from one recirculating system to another or to a purging/finishing system can cause large water imbalances and sump drawdowns in these fairly closed systems if additional piping and pumping networks are not included to allow water flows to remain stable. Because accounting for water flow is critical in water recirculating systems, the technologies to transfer fish and water from growout systems and into finishing/purging systems should be detailed during the design phase.

Unlike gravity-feed fish transfer pipes, this system allows the fish to move up and over equipment, taking advantage of the unused space above the floor and equipment.

Natural Feed Additives Enhance Rainbow Trout Performance

Plant-based additives can take the place of antibiotics in feeds for rainbow trout.

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Summary:
Various additives, many of plant origin, have been explored to replace the use of antibiotics in aquafeed. The authors compared the performance of rainbow trout fed diets containing oxytetracycline or yellow loess and song-gang stone powder, two naturally occurring mineral additives. The results of their study indicated that inclusion of yellow loess or song-gang stone powder at a level of 0.4% in rainbow trout diets could improve growth as well as disease resistance.

The treatment and prevention of diseases in aquaculture through applications of antibiotics and other chemotherapeutants have been responsible for multiple negative impacts, such as an increase in bacterial-resistant pathogen strains. The presence of chemical residues, particularly from antibiotics, in aquaculture products has also led some consumers to consume less seafood.

However, there still is a need for cost-effective and environmentally friendly feed additives that can improve growth and serve as immune stimulators. Various plant products, including herbs, roots, fruits and seeds, have been reported to stimulate appetite and promote growth, act as immune modulators, and have antibacterial and antiparasitic properties in fish and shellfish.

Yellow Loess, Song-Gang Stone Powder

Yellow loess is a naturally occurring product that contains silicon dioxide and aluminum oxide as its main constituents. Song-gang stone is a natural mineral substance predominantly comprised of iron oxide. These products have long been used as supplements in livestock diets for their positive effects on animal growth performance as well as immunity.

Farmers in Korea are known to use yellow loess and song-gang stone powder as feed additives. They have reported improved growth, as well as flesh quality in broiler chicks, and enhanced disease resistance in Holstein calves. Yellow loess and song-gang stone have also been used with fish.

Recently, yellow loess was found to improve the growth of Korean rock fish, *Sebastes schlegeli*. Increased resistance to disease has also been recorded in olive flounders fed diets containing 0.5% song-gang stone powder. Inclusion of dietary yellow loess at a level of 0.4% was also identified as an alternative to oxytetracycline and amoxicillin use in Japanese eels.

These and other claims by various researchers about the potential benefits of yellow loess and song-gang stone powder as a feed additive triggered the authors' interest to investigate their effects in rainbow trout, *Oncorhynchus mykiss*, one of

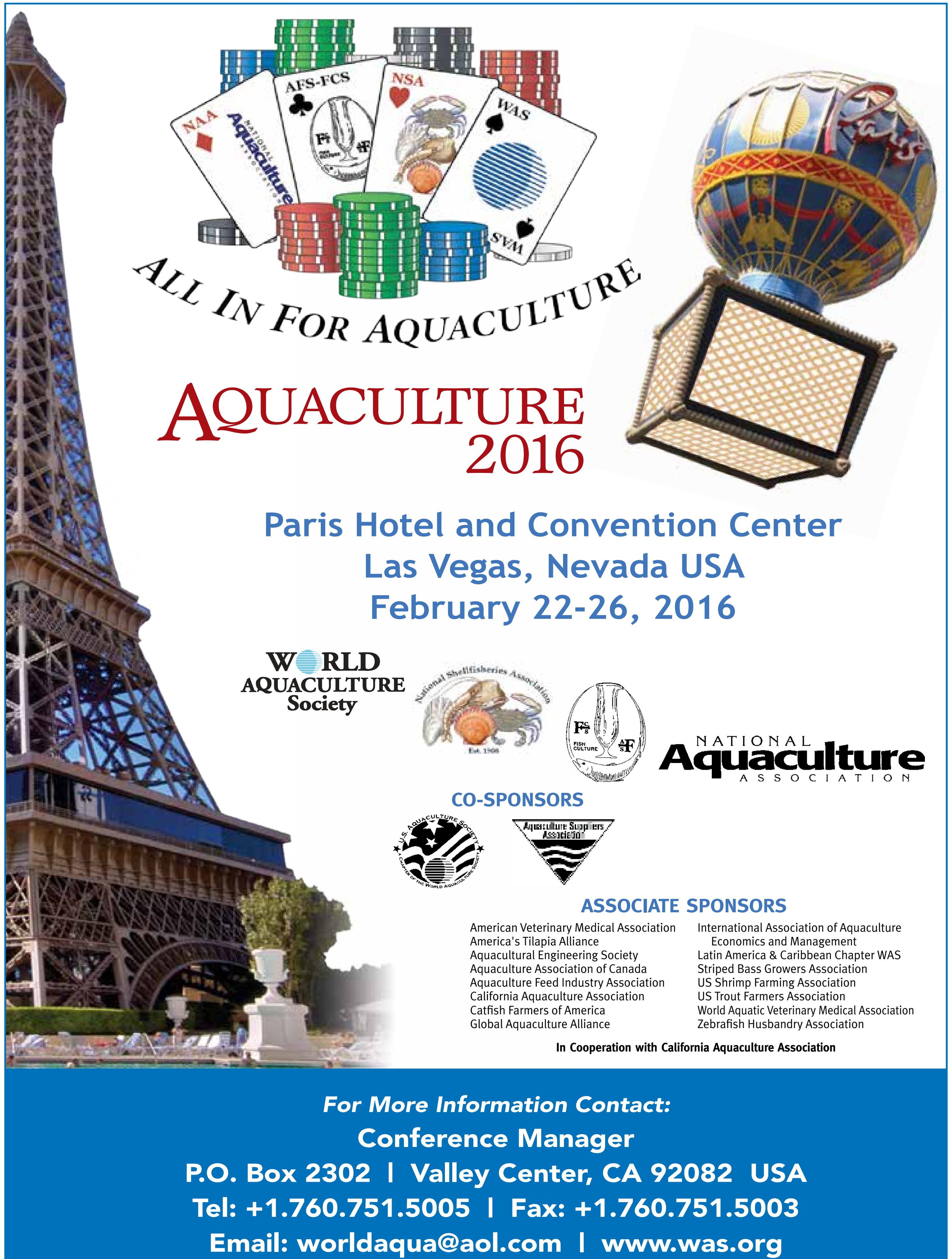

Table 1. Mean growth performance of rainbow trout fed different experimental diets for 22 weeks.

Parameter	Diet: Control	Diet: Song-Gang Stone	Diet: Yellow Loess	Diet: Oxytetra-cycline	Pooled Standard Error
Initial wet weight (g)	258	258	261	259	0.01
Final wet weight (g)	614	659	666	650	11.5
Weight gain (%)	138^{b}	155^{a}	155^{a}	150^{ab}	4.12
Specific growth rate (%)	0.73^{b}	0.79^{a}	0.79^{a}	0.77^{ab}	0.01
Feed efficiency (%)	53.2^{b}	60.1^{a}	60.8^{a}	58.6^{ab}	1.73
Protein efficiency ratio	1.24^{b}	1.40^{a}	1.41^{a}	1.36^{ab}	0.04
Survival (%)	85.6	85.7	87.2	88.8	0.75

Values in each row with different superscripts are significantly different ($P < 0.05$).

the major commercial freshwater species produced in Korea.

Like other fish species in commercial aquaculture, rainbow trout are usually reared in enclosed spaces, and efforts have been made to increase productivity per unit space. This development has led to overcrowding, which tends to adversely affect the health and performance of the fish.

Feeding Trial

The authors conducted a feeding trial over 22 weeks at the Ewhajung fish farm in central Korea to evaluate the use of dietary yellow loess and song-gang stone powder as replacements for oxytetracycline in feeds for rainbow trout. Fish with average initial weights of 261.5 ± 3.5 g were stocked in concrete tanks with recirculation systems and fed 1% of their body weights twice a day.

Experimental diets were formulated to contain yellow loess at 0.4% of the diet, song-gang stone powder at 0.4% of the diet or oxytetracycline at 0.4% of the diet. A control diet was prepared with none of these additives.

Temperature, pH and other water quality parameters were kept at optimum levels during the experimental period. Feeding levels were adjusted every two weeks according to the weight gain.

Results

At the end of the feeding trial, fish that were fed the diets containing yellow loess and song-gang stone powder showed better growth performance compared to the control group (Table 1). Significantly ($P < 0.05$) higher weight gain and specific growth, and better feed conversion were exhibited by these fish.

However, significant differences in these parameters were not observed between fish on the diet containing oxytetracycline and those on the control diet. No significant difference in growth performance was observed among the trout fed the diets containing yellow loess, song-gang stone powder or oxytetracycline.

Protein efficiency ratios were better in the treatments with yellow loess or song-sang stone powder. Survival rates were not affected by the dietary treatments.

Efforts have been made to increase productivity at trout farms.

Development has led to overcrowding, which tends to adversely affect the health and performance of the fish.

Indoor-Raised Shrimp Find Potential Market In Kentucky State University Test

The tank at KSU raised shrimp to over 24 g in 98 days.

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Summary:
By raising shrimp in a closed building, producers can increase biosecurity, produce shrimp more consistently, grow shrimp year-round and locate production centers near markets. Shrimp raised in an indoor recirculating system in Kentucky, USA, grew well with efficient feed conversion. Chefs and consumers were very accepting of the whole fresh shrimp offered at a farmers market. Their survey responses indicated they would pay attractive prices for fresh shrimp, which may be profitable despite the varied costs associated with indoor culture.

Indoor shrimp production is growing in popularity in some parts of the world, including the United States. By growing shrimp in a closed building, producers can dramatically increase biosecurity, produce shrimp more consistently, grow shrimp year-round and locate production centers near markets.

Biosecurity is enhanced through restricted access and controlled inputs. Indoor systems experience minimal water quality variations in temperature, dissolved-oxygen or pH levels. Furthermore, if a consistently productive system can be developed, it may be sited nearly anywhere and sized for the markets it supplies. This may fit well with local foods movements, allow live animal distribution and reduce transportation costs, allowing farmers to receive substantial prices for their products.

In an effort to support industry involvement in this growing field, researchers at Kentucky State University (KSU) are exploring production and marketing aspects of indoor shrimp production.

RAS Culture

This work was conducted inside the newly equipped Aquaculture Production Technologies Laboratory at KSU. The 1,300-m^2 lab building houses a variety of recirculating aquaculture systems. The air temperature of the building is maintained at approximately 23° C year-round using electric boilers.

A 3.1-m^3 rectangular raceway was used as a nursery and outfitted with an external 190-L settling chamber and a 190-L biological filter filled with plastic biomedia. Ten-day-old postlarvae of Pacific white shrimp, *Litopenaeus vannamei*, were obtained from a hatchery in Florida, USA, and stocked into the nursery at a density of 2,500/m^3.

After the nursery phase, 5,250 shrimp weighing an average of 0.55 g each were stocked in a 20-m^3 fiberglass growout raceway with water containing artificial sea salt at a salinity of 20 ppt.

The raceway was equipped with a 1-hp pump, which delivered water to three aeration nozzles distributed around the raceway and one nozzle that fed a foam fractionator.

The nozzles inside the raceway drew air in through snorkels extending above the water surface and directed water around a central wall. The fractionator nozzle delivered finely aerated water into the foam fractionator, which was used as needed to maintain turbidity at approximately 40 nephelometric turbidity units.

Two 3,000-watt submersible heaters in the growout raceway maintained a water temperature of approximately 28.5° C. A dry shrimp probiotic was initially added to the water over a two-week period, and 3 L of biomedia from the nursery was placed in a mesh bag and submerged in the water to help establish a bacterial community.

Results

The growout tank was managed as a biofloc system, with the only external filtration being the foam fractionator to remove dissolved and suspended solids. There was a 1.3 mg/L spike of total ammonia nitrogen one week after stocking shrimp, and a 3.5 mg/L spike in nitrite-nitrogen concentration one month later. Neither spike resulted in noticeable mortality. Sodium bicarbonate was used to maintain pH, which got as low as 7.0. The aeration system was effective at maintaining dissolved-oxygen levels above 6.5 mg/L.

There was 15 cm of tank freeboard above the water surface and 46 cm of vertical netting surrounding the tank. Regardless, 457 shrimp jumped out of the growout tank during this project, mostly on two occasions. Netting was then placed tightly over the top of the tank, which prevented shrimp from escaping. Lights turned on and off in the building may have startled the shrimp, resulting in much of the jumping.

Shrimp were grown in the growout tank for 98 days. Shrimp weighed 24.3 g at harvest and there were 91.8 kg harvested. Survival was 69.1%, although adding the shrimp that jumped out of the tank would have made the survival 80%. The feed-conversion rate was 1.3:1, and the growth rate was 1.7 g/week. The shrimp performance is summarized in Table 1.

Harvested shrimp were sold at a farmers market in Frankfort, Kentucky, USA, where farmers are able to sell a variety of products directly to consumers. Frankfort has a population of approximately 27,500 residents, who had a mean annual per-capita income in 2013 of U.S. $24,100. The shrimp were sold fresh on ice at $26.40/kg. A total of 37.2 kg were sold at this market in only an hour and a half. Also, samples of shrimp were cooked and offered to patrons at the market.

Shrimp were dispersed to chefs in Louisville, Kentucky, through two distribution centers. Shrimp were also given to two chef/restaurant owners and a grocery store in Lexington, Kentucky.

Table 1. Shrimp performance during the first production trial.

Parameter	Value
Final weight (g)	24.3
Growth rate (g/week)	1.7
Biomass (kg/m^3)	4.6
Feed-conversion ratio	1.3
Survival (%)	69.1

Recirculating aquaculture techniques are being developed at the KSU Aquaculture Production Technologies Laboratory.

Survey

Consumers and chefs who tried the shrimp were given a questionnaire. In total, five chefs and 27 consumers completed questionnaires. Their responses are outlined in Table 2.

The questions asked participants their opinions of the Kentucky-grown shrimp regarding taste, texture, freshness, size, overall and appearance. For each topic, the respondents could select: 1 = loved it, 2 = liked it, 3 = it's okay, 4 = disliked it or 5 = hated it.

Respondents were also asked what price they would expect to pay for the fresh whole shrimp, as well as the most they would pay for them. Their options were "will not buy," U.S. $17.60/kg or less, $19.80/kg, $22.00/kg, $24.20/kg, $26.40/kg, $28.60/kg, $30.80/kg, $33.00/kg, $35.20/kg, $37.40/kg, $39.60/kg, $41.80/kg and $44.00/kg or more.

Table 2. Mean responses from chefs and consumers who tasted the shrimp.

Question Topic	Chefs	Consumers
What is your opinion of the shrimp for:		
Taste	2.0 ± 0	1.3 ± 0.1
Texture	2.2 ± 0.5	1.3 ± 0.1
Freshness	1.0 ± 0	1.0 ± 0
Size	2.2 ± 0.2	1.3 ± 0.1
Overall	2.2 ± 0.2	1.1 ± 0.1
Appearance	1.8 ± 0.2	1.1 ± 0.1
What price would you expect to pay? (U.S. $/kg)	21.6 ± 2.4	25.9 ± 2.3
What is the maximum you would pay? (U.S. $/kg)	26.0 ± 2.5	28.6 ± 1.5

Responses were rated 1 to 5, with 1 the best rating.

Costs

The recurring costs of nursery and growout production for this project were approximately U.S. $12.10/kg. Calculated at $0.04/kWh, electricity fees accounted for 25% of production costs. Labor at $10.00/hour accounted for 28%, while feed reflected 29% of the total costs. Postlarvae at 16% and other consumables at 2% made up the remainder of the total.

If 14 growout tanks were used, one tank could be harvested weekly all year. If more tanks were used, an economy of scale effect should be realized, bringing down costs. Other cost considerations include heating the air, infrastructure, taxes and distribution. These should be considered carefully and vary depending on a farmer's circumstances.

Perspectives

Shrimp grew very well during this project. They had an efficient feed conversion, and a substantial amount of the mortality that occurred could easily be prevented. Survey respondents were very accepting of the product and scored it highly. Consumers appeared willing to pay more than chefs, possibly because chefs are motivated more by the profitability of their restaurants.

The highest-scoring attribute was freshness of the product, a quality that cannot easily be achieved without year-round, indoor, local shrimp production. A direct-to-consumer approach for shrimp sales could prove to be profitable. At a sale price of U.S. $26.40/kg, there appears to be room for profit, and according to survey responses, a higher price may be acceptable to consumers.

Future efforts at KSU will focus on increasing shrimp stocking density and survival to enhance production output and augment the potential profitability of this approach. The sale of live shrimp will also be explored.

Metabolomics Approaches To Improve Mussel Larval Production

Poor-quality mussel larvae (left) grow more slowly than higher-quality – and faster-growing – larvae (right). Scale bars = 20 μm. Photos by A. Rusk.

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Summary:
Variability in the quantity and quality of larval yields limit aquaculture growth. However, new biotechnological advances promise to revolutionize the way we assess and solve bottlenecks. A study demonstrated the use of metabolomics, which provide a snapshot of larval physiology through metabolite profile analysis, to assess and classify mollusk larvae quality and identify biochemical pathways that may reveal further important insights. Analyses of metabolites and their ratios can be integrated with gene and protein expression data to provide new avenues for selective-breeding programs.

Hatchery production of mussel larvae presents a number of biological and technological challenges that continue to hamper growth within the industry. Unexpected batch crashes are routinely observed in some hatcheries, and the causes are often not identified. When solutions are found and improvements made, these tend to be incremental and directed by technical managers.

Unfortunately, technical developments are poorly captured in the primary scientific literature and often not shared due to concerns over losing commercial advantage. In general, low production yields are hampered by high larval mortality rates that stem from the poor quality of broodstock, inadequate feeding regimes or unhealthy culture systems.

With the complex physical and biological factors within the larval-rearing process, it is not surprising larval cultivation still remains a hit-and-miss approach with variable chances of success. In this regard, metabolomics can provide a powerful means of identifying problems, and potentially could be used as an early warning system in subsequent cultures.

Metabolomics

Metabolomics is the study of chemical processes involving metabolites. As Wikipedia said, metabolomics is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind." Metabolic profiling can give an instantaneous snapshot of the physiology of cells.

Because this is a new application in larval biology, there are only a few studies to illustrate its advantages for aquaculture. The authors recently performed a study using metabolomics to investigate intraspecific growth variations in New Zealand Greenshell™ *Perna canaliculus* mussel larvae.

Mussel Aquaculture In New Zealand

In New Zealand, exports of *P. canaliculus* mussels represent the largest aquaculture sector by value and volume. Small-scale hatchery production of spat currently contributes only marginally toward the industry's seed requirements. However, substantial research over the past decade has led to the ongoing development of selective breeding lines, establishment of a cryopreservation program, optimization of microalgae culture and larval-rearing procedures, and future strategies for extensive growth and upscaling of hatchery facilities.

The ability to provide consistency in larval quality and quantity is a critical step to achieve the commercial goal of successful large-scale production. Identification of biomarkers that reflect the immediate physiological condition of larvae has the potential to provide valuable tools that not only determine larval batch viability early on, but also identify the causes of poor production levels. This information can lead to better management decisions and improve overall hatchery operations.

Study Setup

In this study, the physiological condition of mussel larvae was used to assess larval quality during hatchery production. Mussel larvae were grown to umbo stage, separated based on relative sizes to divide poor-quality, slow-growing larvae from high-quality, fast-growing animals, and sampled over time. Six samples including about 80,000 pooled individuals from each of the two size fractions and three replicate rearing tanks were used for metabolite extractions via gas chromatography/mass spectrometry.

Metabolite peaks were identified, and their relative abundances were calculated.

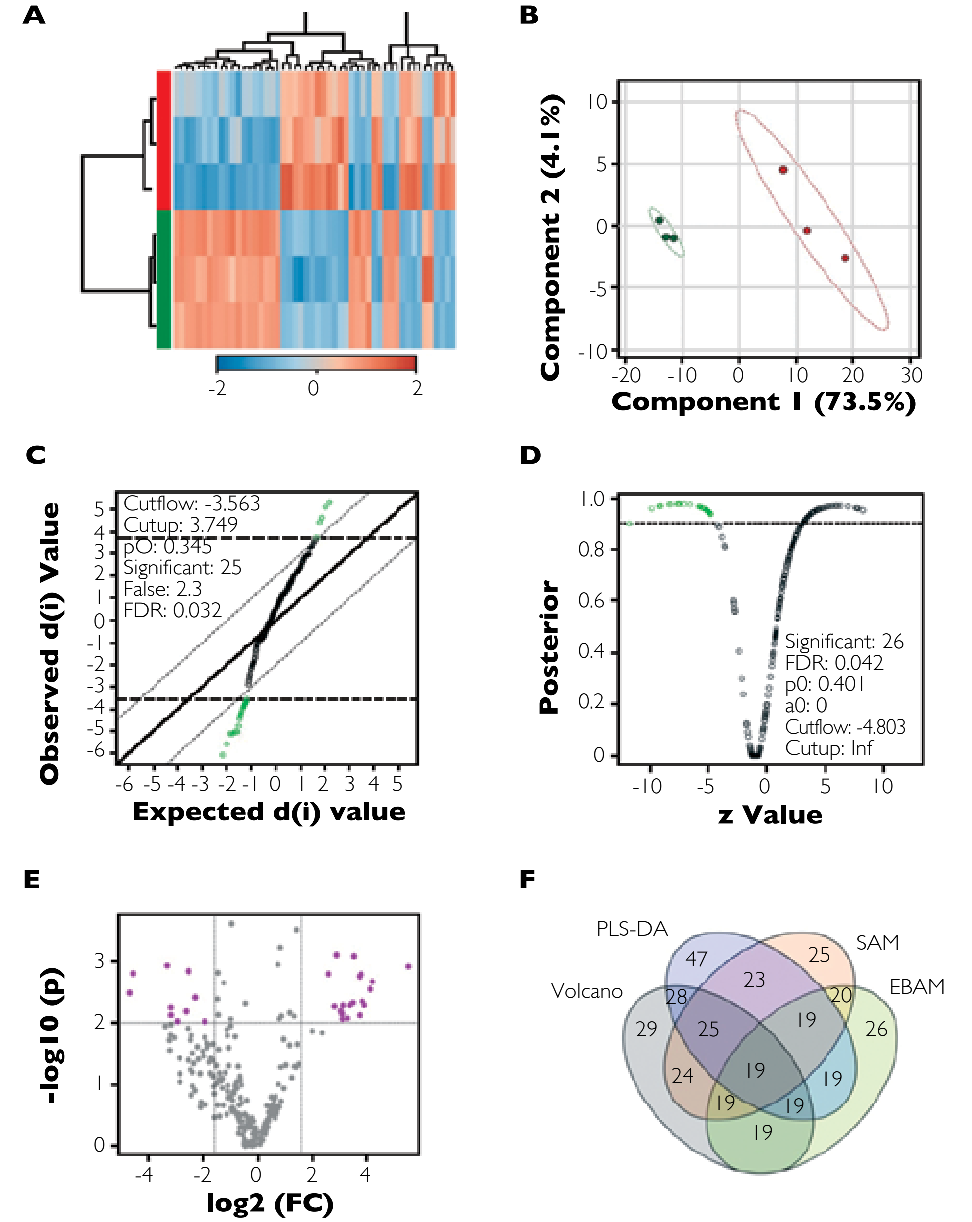

Figure 1. PLS-DA scores plot (B) of poor (circles) and high (squares) quality larvae. Hierarchical clustering and heat map (A) of poor (■) and high (■) quality larvae based on the top 50 features ranked by their T-test statistic (samples on the y-axis, features on the x-axis). PLS-DA scores plot (B) of poor- (●) and high- (●) quality larvae. SAM plot (C) of significantly different features (○) between quality classes (upper right = ratios that were higher in poor-quality group, lower left = ratios that were lower). EBAM plot (D) of significantly different features (○) between quality classes (all lower in the poor-quality group). Volcano plot (E) of features (●) with a between-class fold-change greater than 5 and a T-test statistic below 0.01 (upper left = ratios that were higher in the poor-quality group; upper right = ratios that were lower). Venn diagram (E) displays the counts of commonly identified ratios by the four independent feature selection methods.

The dataset matrix was subjected to a variety of feature selection methods to identify potential biomarkers for discrimination of larval quality classes. Comprehensive Web-based analytical pipeline tools were used to statistically analyze the data sets.

To visualize feature differences between the larval quality classes, metabolite ratios were used to perform agglomerative hierarchical cluster analysis, displayed in dendrograms and heat maps. Four methods of feature reduction were used independently to minimize selection bias and provide robust criteria for assisting candidate biomarker identification.

Results

From the initial 253 metabolite ratios analyzed, candidate biomarkers were identified for assessing mussel larval quality using four independent feature selection methods: Volcano plot, partial least squares discriminant analysis (PLS-DA), significant analysis of microarrays (SAM) and empirical Bayesian analysis of microarrays (EBAM). These methods resulted in the selection of 19 common features.

Based on their performance, these were reduced to a final four metabolite ratios that were good candidates for assessing larval quality: alanine/succinate, glycine/succinate, myristic acid/succinate and pyroglutamate/succinate. Each of the identified ratios was substantially lower in the poor-quality larvae (Figure 1).

Comparing this group with the faster-growing cohort, the trend was characterized by elevated levels of succinate and simultaneous reductions in relative metabolite abundances of alanine, glycine, pyroglutamate and myristic acid. Based on the known functions of these metabolites, it was possible to identify potential biochemical pathways involved in larval performance. These pathways include energy metabolism, osmotic regulation, immune function and cell-to-cell communication.

Perspectives

While this study identified broad areas of larval physiology responsible for larval quality, the authors' ongoing studies are providing more specific biomarkers intended to generate predictive models of larval performance. Furthermore, the aim is to eventually develop easy-to-use tool kits to evaluate the physiological state of larvae throughout the rearing process.

Further analysis of the single metabolites and their ratios will likely reveal additional information, and when integrated with gene and protein expression data, could provide new avenues for selective-breeding programs to consistently yield high-quality larvae. Supplementary experiments incorporating metabolomics-based approaches to investigate other measures of larval quality – such as health after immunological challenge and nutritional condition in response to diet manipulation – have the potential to offer a suite of biomarkers for a range of applications.

The use of metabolomics could also be applied to other areas of hatchery production to help close the loop on full life-cycle culture for many species. For example, routine production of high-quality gametes for successful fertilization and ongrowing could be achieved through better broodstock management and understanding of maternal provisioning, paternal effects and factors associated with high fecundity.

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