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“Sustainable Development of Cage Aquaculture in Asia”

“亚洲网箱养殖可持续发展”

# Abstract Book

# 摘要集

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# **Session 1 Production Systems**

## **专题一：养殖系统**

## **Cage Farming in Tropical Estuaries Along The Southwest Coast of India: Empowering Traditional Fishers with Alternate Livelihood Options**

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**Abstract:** The decline in subsistence fishing along the Indian coast is attributed to the salinization of estuaries, resulting from both human activities such as water diversion and the rising sea levels due to climate change. This has adversely affected estuarine coastal communities, depriving them of their livelihoods. To address this issue, small-scale cage farming has been introduced in coastal villages, offering an alternative livelihood and enhancing fish production. Saline creeks and estuaries along the Indian coasts offer opportunities for culturing euryhaline species through the installation of small cages. Cage-based aquaculture is a widespread practice globally. Since 2006, the Central Marine Fisheries Research Institute (CMFRI) has successfully initiated the cultivation of marine finfish in cages in several maritime states. Notably, the state of Karnataka stands out for its initiative in promoting cage farming among traditional coastal fishers. This participatory approach has not only exposed local fishers to the intricacies of finfish rearing but also raised awareness about this profitable farming technique. Encouraged by this success, numerous fishermen groups have come forward in cultivating finfish in suitable areas near their homes, leading to the proliferation of small-scale finfish culture along the coast. Starting with 5 cages, which yielded about 1.8 tons of fish in a 10-month cycle, strategically timed for the lean fishing season in fishing not only supplemented income but also strengthened traditional fishing communities, offering an alternative to estuarine fishing. Currently, the coastal region boasts more than 1000 installed cages, underscoring the broad acceptance of this innovative approach. Over the years, the constant pursuit of improvements in cage design and operational techniques has directly led to a significant increase in fish production through cage farming. This evolution is particularly evident in the quantitative shift, with the initial yield of 1.8 tons in 2009-10 surging to an impressive 800 tons during the progressive period from 2022-23. This substantial increase has contributed Rs 40 million (\$4800000) to the state's overall fish production. The successful proliferation of this technology has been accomplished through a holistic strategy, which includes participatory methodologies, focused group discussions, expert-led training sessions, and practical technical guidance. Over the last fourteen years, the small-scale cage farming initiative has delivered significant results, characterized by a remarkable increase in fish production and a concurrent improvement in the socio-economic status of fishing communities. The concrete results of this effort are plainly visible: the adoption of advanced techniques, heightened production rates through strategic farming, and the empowerment of fishers. The viability of this technology has been consistently upheld, fostering a dynamic environment that has encouraged continuous refinement and

diversification of the species being cultured viz; seabass, snapper, pearl spots, and recent inclusion of Pompano among the successfully cultured species within these cages. In conclusion, the ongoing endeavors of cage farming in coastal waters have not only provided alternative livelihood opportunities but have also enabled a meaningful diversification of income sources for traditional fishers in the state. These initiatives are simultaneously addressing the urgent issues of nutritional and financial security within coastal fishing communities.

**Keywords:** coastal waters; coastal communities; CMFRI; finfish farming; income generation.

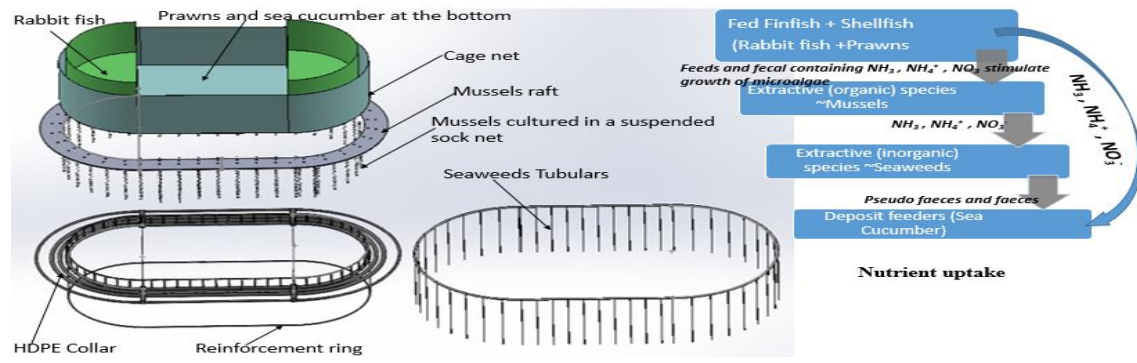
## Design of an Advanced Offshore Cage Culture System for Sustainable Integrated Multitrophic Aquaculture

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**Abstract:** Marine and coastal aquaculture have excellent potential in increasing seafood supply which would reduce malnutrition and hunger in the growing population around the globe. Traditional reliance on freshwater systems, wild capture fisheries, and imports is deemed inadequate due to climate change-induced disruptions affecting water stability and migratory patterns of wild species. Large-scale open sea Integrated Multi-Trophic Aquaculture (IMTA) in an offshore cage system is proposed as a viable solution to achieve food security goals while minimizing environmental impact, given its low greenhouse gas emissions. Despite the recognized benefits of offshore aquaculture, challenges arise from harsh oceanic conditions, necessitating the development of resilient aquaculture cage designs. Notably, limited attention has been directed toward integrating IMTA principles for optimized nutrient utilization within cage design frameworks. In this research, a novel stratified hybrid cage is introduced, designed using computer-aided engineering (CAE) software, and subjected to computational fluid dynamics (CFD) finite element (FE) modeling to assess its structural response under diverse oceanic conditions. The proposed cage design demonstrates effectiveness in minimizing structural failure and fish escape, enhancing fish welfare, and optimizing nutrient utilization. The study extends its focus to the integration of seaweed tubular cultivation within the aquaculture system as a means of enhancing global carbon sequestration efforts. This cultivation method is depicted as contributing to a scientifically robust and sustainable framework for mitigating carbon emissions on a global scale by capturing, utilizing, and storing carbon in diverse molecular forms. Therefore, the future of sustainable aquaculture is envisioned in offshore waters, presenting untapped land resources relatively free from significant pollution. This study underscores the necessity for innovative cage designs capable of withstanding hydrodynamic loads and proving economically viable for aquaculture farmers. This investigation offers a valuable foundation for intensive cage aquaculture in offshore environments, promising to unlock unexplored benefits of the ocean while addressing global food security challenges.



**Keywords:** Offshore; Aquaculture Cage; Seaweed Tubular

## Sea-Land Relay Culture Technology for Fish Farmed in Large Offshore Intelligent Aquaculture Cage in China

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**Abstract:** In recent years, we designed and constructed eight large deep-sea intelligent aquaculture cages named "Jinghai 001-008" in China, with a water capacity of up to 70,000 cubic meters and a maximum operating draft of 36.5 meters, capable of withstanding the impact of category 12 typhoons. The power supply mode for the cages utilizes wind-solar energy storage complementarity. In addition, the cages was equipped with automated pneumatic bait feeders, net winches, net washing machines, underwater three-dimensional vision monitor systems, multi-beam sonar systems, net clothing health detection robots, and other automation equipment. Additionally, an advanced marine aquaculture intelligent management platform system has been developed to achieve automation, digitalization and intelligence in open ocean aquaculture practices of marine fish. Furthermore, marine fish species suitable for these intelligent large cages culture have been selected while key technologies such as large-size seed cultivation techniques have been developed along with land-sea relay transport methods, precise feeding strategy, stress reduction measures, fish face identification technology, automatic cleaning technology, etc., which provided valuable technical insights for the development of large-scale farming facility culture in open ocean. This report also addressed existing challenges faced by open ocean culture operations using large-scale cages, and provided the potential solutions, furthermore, the future development prospects was discussed. All the information could be served as a reference for the advancement of open ocean aquaculture industry in China.

**Keywords:** Intelligent aquaculture cage; Marine fish; Open Ocean Aquaculture; Land-sea relay culture technology

## Study of Automatic Control and Management System for Sinking and Floating Aquaculture Cages

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**Abstract:** Aiming at the problem that the inclination angle is too large and easy to roll over when the sinking and floating aquaculture cages is sinking and floating. In this study, an automatic control and management system for sinking and floating aquaculture cages was developed. The automatic control system of sinking and floating aquaculture cages is mainly based on PID control technology. In order to realize automatic control of the inclination angle of cages, and the appropriate parameters of the PID control system are explored, which uses inclination angle signal and control algorithm to adjust the actuator. The corresponding management system for automatic control of sinking and floating aquaculture cages was developed. The main functions include: floating and sinking control, feeding control, cleaning cage, GPS positioning and camera cleaning, as well as underwater camera monitoring, water quality, flow rate, liquid level and pressure data monitoring, etc. After a series of experiments such as the buoyancy-gravity ratio of the cage, the water inlet diameter and diameter ratio of the adjusting buoy, the PID control, the suitable values of the buoyancy-gravity ratio of the cage is 1.2, the water inlet diameter and diameter ratio of the adjusting buoy is 0.03, and the PID parameters are P:100, I:1000, D:0. In this study, the control and information of each module of the sinking and floating aquaculture cages were integrated into a set of system, which improved the performance of the automatic control system of the deep-sea aquaculture cages. The system can control the output in a timely and stable manner, and achieve the stable sinking and floating of the aquaculture cages. The results of the research have certain theoretical and practical meaning. The automatic control system of deep-sea sinking and floating aquaculture cages is one of the important technical means to achieve the industrialization development of deep-sea aquaculture. The research methods and experimental results proposed in this paper provide important data reference and theoretical basis for the research and application of deep-sea sinking and floating aquaculture cages.

**Keywords:** Sinking and floating aquaculture cages; Automatic control; Management system; PID control

# Development of Large-scale Vacuum Fish Pump and Its Application on Deep Sea Aquaculture Engineering Equipment

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**Abstract:** At present, the space of mariculture in China has gradually expanded from near shore to deep sea, and the deep sea aquaculture has developed rapidly. Large-scale, automatic and intelligent deep sea aquaculture engineering equipment is the key to carry out deep sea aquaculture. The main way for developed countries in the world to develop deep sea aquaculture engineering equipment is to build large-scale aquaculture cages and floating aquaculture platforms, and the floating aquaculture platform is represented by specialized aquaculture vessels. As modern fishery equipment, fish pump plays an important role in catching fish and has been widely used at home and abroad. The vacuum fish pump (VFP) has the advantages of high automation, simple structure, high working efficiency, no moving parts, no damage to fish, and suitable for transporting large-scale live fish, so the VFP is ideal mechanized fishing equipment for deep sea aquaculture engineering equipment. The author started the development of the VFP in 2017. After years of continuous research, the technological process and control logic were mastered, and the process calculation program was developed. Single-tank and double-tank VFP prototypes were designed and manufactured successively, and the performance tests were carried out, through which the performance law and key parameters were mastered. The numerical simulation of the VFP was also carried out using computational fluid dynamics method. The numerical simulation results were consistent with the VFP prototype test results, which proved that the numerical simulation method was correct. The numerical simulation research provided theoretical basis for computer-aided design of the VFP. Those research findings laid a solid theoretical and technical foundation for the design of large-scale VFP. Four large-scale VFPs were designed for Conson No 1 in 2020, which is the world's first 100,000-metric-ton smart aquaculture vessel. Those four VFPs passed the shipowner's acceptance in June 2022, and then were put into use. Up to now, the four VFPs have successfully accomplished several harvests of adult *Larimichthys crocea*, with reliable performance and high work efficiency, and the caught *Larimichthys crocea* had no damage. The application effect of the four VFPs has been highly recognized by the shipowner, and therefore a new supply contract of large-scale fish pumps for Conson No 2 and No 3 was signed in May 2023. The successful application of the designed VFPs on Conson No 1 lays a solid foundation for the design of large-scale VFPs for deep sea aquaculture engineering equipment.

**Keywords:** Deep sea aquaculture engineering equipment; Large-scale vacuum fish pump; Cage; Aquaculture vessel

## New Connection Structure for the Metal Fish Net

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**Abstract:** This utility type of copper alloy mesh connection solution is a stronger way to enhance the string of fish net after being improved, plus the spiral wire to suture two or more nets. The characteristics are: more reverse hook edges make the lock more firm, and there will be no edge cracking or removing wires. The addition of two straight string makes the spiral wire wound. The sewing connection is more accurate and reliable. This kind of new type of hexagonal mesh connection device can further improve the structure system of the network, make the cage more secure, more reliable. Reduce fish escape and the invasion of foreign predators, reduce the edge of net clothes damage, thereby reducing the frequency of underwater maintenance of the net, saving manpower and material resources, and further reduced maintenance costs for deep sea breeding.

**Keywords:** Copper alloy; Fish net; Copper Wire Aquaculture farm fish net; off-shore copper alloy fish net

## Study on the Regulation of Aquaculture Water Quality in a Circulating System Using Microecological Preparations

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**Abstract:** With the aim of promoting sustainable development in aquaculture, microecological preparations have garnered increasing attention as a green and pollution-free alternative to traditional antibiotics. The present study aimed to investigate the removal efficiency and the influence of inoculation time (The treatment lasted for 10 days, and the water quality index was measured every 2 days) and amount (1.5、3、4.5 ml) on aquaculture water quality indexes using three commercially microecological preparations, including *Bacillus subtilis*, *Lactobacillus spp.*, and *Rhodopseudomonas palustris*. The results revealed that the removal rates of NH<sub>3</sub>-N varied with the inoculation time for *B. subtilis*, *L. spp.*, and *R. palustris*. After approximately 6 days of inoculation, *B. subtilis* exhibited the highest NH<sub>3</sub>-N removal rate (51.23%), while *R. palustris* achieved the highest removal rate (26.50%) on the 8th day of inoculation. *Lactobacillus spp.*, however, showed limited effectiveness in removing NH<sub>3</sub>-N and NO<sub>2</sub><sup>-</sup>-N, with no significant change observed as the inoculation time increased. The removal rates of NH<sub>3</sub>-N and NO<sub>2</sub><sup>-</sup>-N by *B. subtilis* and *R. palustris* exhibited an increase with the inoculation amount. Specifically, when 4.5 mL/L of bacterial solution was used, *B. subtilis* achieved a removal rate of 61.20% for NH<sub>3</sub>-N, while *R. palustris* achieved a rate of 44.54%. For NO<sub>2</sub><sup>-</sup>-N, the corresponding rates were 40.86% for *B. subtilis* and 28.49% for *R. palustris*. *Lactobacillus* did not exhibit significant improvement in its ability to remove NH<sub>3</sub>-N and NO<sub>2</sub><sup>-</sup>-N as the inoculation amount increased, with maximum removal rates reaching only 5.74% and 6.99%, respectively. The optimal inoculation amount and time for *B. subtilis* resulted in a superior COD removal rate (24.27%), significantly higher than that observed in other groups ( $P < 0.05$ ). It also had the highest TN removal rate (15.69%), but there was no significant difference compared to other experimental groups ( $P > 0.05$ ). All microecological preparations had limited removal rates for TP, with no significant difference compared to the control group ( $P > 0.05$ ). The findings of this study contribute to the practical application of the three microecological preparations in aquaculture and offer valuable insights for water quality regulation in circulating system.

**Keywords:** microecological preparations; circulating system; *Bacillus subtilis*; *Lactobacillus spp.*; *Rhodopseudomonas palustris*

## Design and Experimental Study of a Cleaning Device For an Aquaculture Net Cage

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**Abstract:** With the increasing global demand for seafood, cage culture is also expanding the scale of farming, but cage culture because of the long-term immersion of net clothing in seawater is easily attached by shellfish, algae and other organisms, seriously affecting the exchange of water quality in the cage, resulting in frequent fish diseases and other problems. Considering the current situation of aquaculture, this paper studies and proposes an in-situ cleaning device for cages. A combination of brush and rotary cutter head high pressure water jet cleaning technology is used to clean the objects attached to the cage, and the removable cage frame is used for daily manual maintenance of the organisms in the cage. The nozzle structure and key design parameters of the cleaning unit have been optimised using Fluent fluid simulation technology. Through orthogonal experiments, it is found that when the throat length is 3mm, the outlet diameter is 1.4mm and the outlet angle is 45°, the optimum jet parameters of the mutant nozzle are obtained, and the gas phase volume fraction of the nozzle is up to 85%, which can greatly improve the cleaning efficiency. Solidworks was used to simulate the effect of the speed of the fishing vessel on the spiral motion of the cage driven by the brushes. Design and trial production of cage cleaning equipment test rigs, conduct orthogonal tests to verify the feasibility of cleaning methods, and provide theoretical basis and technical support for research and development of mechanised mesh cleaning equipment for aquaculture cages.

**Keyword:** Aquaculture cage; Attachment; Cavitation jet; Mesh washing

## **Session 2 Breeding and Seed Production**

### **专题二：育种和苗种繁育**

## Applications of Genomic Selection for Complex Economic Traits in Large Yellow Croaker

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**Abstract:** Large yellow croaker is one of the most important mariculture species, with the highest annual mariculture production in China. With the fast development of large yellow croaker culturing, this industry is confronting with a series of problems, such as frequently outbreak of diseases, disruption of marine ecological system caused by consumption of forage fishes, degradation of germplasms. In order to overcome these bottlenecks, we constructed lot of genetic tools including chromosome level genome sequences, genome wide level SNPs, high throughput SNP array, genomic selection models etc. By the utilization of these genetic tools, we conducted genomic selection of resistance against *Cryptocaryon irritans* and *Pseudomonas plecoglossicida*, swimming performance, and highly efficient utilization of plant protein, and the heritability of those traits was calculated. Genome-wide association analysis revealed that these traits were all complex quantitative traits controlled by polygene. The results of infection challenge of F3 generation showed that the survival rate of the resistance against *Cryptocaryon irritans* strain was 40.8% higher than that of unselective line. For the strain resistant to *Pseudomonas plecoglossicida*, we bred it to F2 generation, and the result of offspring evaluation showed that the breeding line was 18.6% higher than the unselective line. The swimming performance of the offspring of the breeding line was 15.8% higher than that of the non-selected line. The growth results of F2 generation of selected line with highly efficient utilization of plant protein showed that the weight gain rate of selected line was 16.3% higher than that of non-selected line. In the future, genetic improvement based on multi-trait selection index of disease resistance, robustness traits will be the focus of genomic selection breeding of large yellow croakers.

**Keywords:** Genomic selection; Phenotypic measurement; Genotype; Disease resistance

## Deletion of Hemolysis-related Genes Attenuated the Pathogenicity of *Vibrio anguillarum*

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**Abstract:** *Vibrio anguillarum* is a pathogenic bacterium widely distributed in seawater and marine organisms. It is one of the important sources of bacterial diseases and can cause serious vibriosis. Hemolysin is an essential component of virulence factor of *V. anguillarum*, which cause hemorrhagic septicemia in the host. To explore the role of the hemolysin on the pathogenicity of *V. anguillarum*, the deletion of three hemolysis-related genes *vah1*, *vah4* and *rtxA1* was thereafter deleted by gene recombination and overlap PCR. The characterization experiment showed that  $\Delta vah1-vah4-rtxA1$  strain of *V. anguillarum* had almost no hemolytic activity, and the biofilm formation ability decreased by 30%. The median lethal dose of  $\Delta vah1-vah4-rtxA1$  strain infecting shrimp *Litopenaeus vannamei* is  $3.24 \times 10^6$  cfu/mL, compared with the wild strain of *V. anguillarum*  $3.16 \times 10^5$  cfu/mL increased by 10.25 times. Compared with the wild strain, the colonization ability of  $\Delta vah1-vah4-rtxA1$  strain in the hepatopancreas of *L. vannamei* was significantly decreased, and the degree of damage to the hepatopancreas cells caused by dissolution and vacuolation was also relatively small, and the expression level of hepatopancreas immune-related genes *cat*, *sod*, *po*, *lzm* and *acp* was reduced to a certain extent. These results showed that the deletion of *vah1*, *vah4* and *rtxA1* affected the pathogenicity of *V. anguillarum* to *L. vannamei*. Moreover, in order to evaluate the pathogenicity of the strain  $\Delta vah1-vah4-rtxA$  of *V. anguillarum* to fish *Lateolabrax maculatus*, the colonization ability of bacteria in *Lateolabrax maculatus*, histopathological changes and immune response of *L. maculatus* after intraperitoneal injection were evaluated. Compared with the LD50 of wild strain  $2.103 \times 10^5$  CFU/mL,  $\Delta vah1-vah4-rtxA$  was  $1.837 \times 10^6$  CFU/mL. The colonizing ability of *V. anguillarum*  $\Delta vah1-vah4-rtxA$  and the damage degree of intestinal mucosa vacuolation and gill necrosis can be reduced as well. The KEGG pathway enrichment analysis showed that the differently expressed genes were significantly enriched into hematopoietic cell lineage, antigen processing and presentation, cell adhesion molecules and intestinal immune network for IgA production signal pathways related to immune response. The above results also showed that deletion of hemolysis-related genes *vah1*, *vah4* and *rtxA1* attenuated the pathogenicity of *V. anguillarum* infection to *L. maculatus*.

**Keywords:** *Vibrio anguillarum*; *Litopenaeus vannamei*; *Lateolabrax maculatus*; hemolysin; Pathogenicity

## Analysis of Pigment Cell Changes and Control Genes Expression in the Embryonic Period Development of Body Color Pattern of *Amphiprion ocellaris*

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**Abstract:** This study recorded 6 developmental stages of the embryonic period of *Amphiprion ocellaris* with significant changes in pigment cells. The changes in body color and pigment cells at different stages were observed, and the expression of 10 body color-related genes at each developmental stage was detected using fluorescence quantitative PCR. The results showed that during the embryonic period, the color of the entire embryo gradually changed from orange yellow to dark red, black red, black, and finally transparent. The transformation process from larval melanocyte to melanocyte on the surface of the yolk sac was observed. Melanocyte appeared on the surface of the fish during the rollover period, erythrophore appeared during organogenesis period, and iridophore appeared in the eyes and abdomen during hatching. Based on the analysis of fluorescence quantitative PCR results, it was found that the larval melanocyte on the surface of the yolk sac may have developed directly from neural crest cells in the ectoderm, without the need for migration; *Ednrb*, *TYR*, and *Tbx2b* genes played an important role in the formation and development of larval melanocyte, while *PAX3*, *DCT*, and *Aim1* genes were closely related to the differentiation, migration, and formation process of melanocyte; the relative expression level of *TYR* gene significantly increased during the segmentation period, indicating that *TYR* gene was involved in the formation of melanocytes during early embryonic development; the *Tbx2b* gene not only affected the formation of melanocytes, but also had a certain effect on the formation of the heart of *A. ocellaris*; The *fms* gene played an important role in the formation of erythrophore; the expression of *Itk* gene was earlier than the appearance of iridophore; correlation analysis revealed interactions between various genes.

**Keywords:** *Amphiprion ocellaris*; development; body color; pigment cells; expression analysis

## Development and Application of Genomic Selection Breeding Techniques for Freshwater Pearl Mussel *Hyriopsis cumingii*

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**Abstract:** Freshwater pearl mussel (*Hyriopsis cumingii*, Lea 1852) is the most important aquaculture species for pearl production. Growth traits of host mussels and inner shell color traits of donor mussels are critical determinants for pearl size and color. In this study, using genotype by sequencing (GBS) technique, we obtained 97,090 SNPs from 764 mussels (496 for reference population, 268 for candidate population) collected from F2 generation of “Shenzi NO. 1” new varieties. Using these data, we performed genome-wide association analysis (GWAS) and genomic selection (GS) for growth and inner shell color traits. The five growth traits exhibit typical quantitative genetic pattern, while four inner shell color traits, especially redness  $a^*$  and color difference  $dE^*$  were likely complex traits controlled by a combination of few major effect loci (cluster on chromosome 17) and numerous minor effect loci. Moreover, GWAS identified 12 growth-related and 29 inner shell color related significant SNPs, respectively. Additionally, we predicted their genomic heritabilities, which were moderately high (0.36–0.67). Then, we compared the effects of GBLUP and four Bayesian models and different number of SNPs (selected using three SNP screening strategies) on genomic predictive accuracy. For growth traits, GBLUP model containing 10,000 SNPs selected using GWAS strategy was the most appropriate and cost-effective, whilst, for inner shell color traits, BayesB model with 5,000 GWAS-informative SNPs was better. Furthermore, we applied these two GS methods in real selective breeding within candidate population. The individuals with high growth GEBVs were selected as broodstock for fast-growth strain (FS) (n=30), high purpleness (50%  $a^*$  + 50%  $dE^*$ ) GEBVs were selected as broodstock for purple strain (PS) (n=30), and the rest for control strains (CS). As expected, FS significantly outperformed CS in terms of growth, with different traits increasing by 5.74–28.77% and 11.93%–54.58% at the Wuyi (WY) and Chongming (CM) sites, respectively. Also, significant differences in  $a^*$  and  $dE^*$  were detected between PS and CS, as well as the proportion of deep purple individuals rose from 9.57% and 12.38% to 51.16% and 26.92% in WY and CM sites, respectively. At last, we constructed a 30 K cGPS SNP array known as “Zhuxin NO. 1”. This array comprises 30,621 high-quality SNPs evenly distributed throughout the *H. cumingii* genome, with an overall accuracy exceeding 97.5%. In conclusion, we have, for the first time, conducted a genome-level exploration of the genetic architecture underlying these two crucial traits and have introduced two GS methods. This advancement not only significantly enhances the genetic improvement of *H. cumingii* but also offers a crucial reference for other aquaculture species.

**Keywords:** *Hyriopsis cumingii*; growth traits; inner shell color traits; genomic selection; SNP array

# Genomic Basis of Pigment Pattern Diversification in *Danio* Species: Implications for Aquaculture and Evolutionary Biology

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**Abstract:** Teleost fishes are notable for generating remarkably diverse pigment phenotypes, which constitute key substrates for natural and sexual selection. However, hereditary color defects, pigmentation trait segregation in hybrids, or melanotic hyperpigmentation can severely undermine the economic returns of aquaculture production. Elucidating the pigmentation mechanisms in fish could provide theoretical guidance and technical support for scientific aquaculture in multiple aspects. The Danioninae subfamily encompasses up to four hundred distinct species that have evolved to exhibit a dazzling variety of pigment patterns, including horizontal stripes, vertical bars, spots or lack a clear pattern. The diverse pigmentation patterns of closely related species within *Danio* offer an excellent model to unravel the genetic mechanisms underlying pigment pattern evolution. We assembled genomes of 14 *Danio* and *Danionella* species. The two sister species *D. rerio* and *D. aesculapii* exhibit divergent patterns of horizontal stripes and vertical bars that are partly attributable to the divergence of the potassium channel gene *kcnj13*. We demonstrate that *kcnj13* is required solely in melanophores for interactions with xanthophores and iridophores. Molecular analyses identified positive selection and structural variations in pigmentation genes *igsf11* and *gja5b*, which were functionally diverged between spotted and striped species, proved by the interspecific complementation tests. Strikingly, convergent evolution occurred in the C-terminal domain of *gja5b* in both spotted species, suggesting recurrent causative changes. Our phylogenomic and functional analyses integrate to elucidate complex genomic changes driving phenotypic diversification in *Danio*. Overall, deciphering the precise molecular events dictating pigmentation will substantially assist quality enhancement and trait optimization in aquaculture regarding coloration.

**Keywords:** Pigmentation; *Danio* genus; Genome assembly; Phylogenetics

## IFN regulatory factor 3 of golden pompano and its NLS domain are involved in antibacterial innate immunity and regulate the expression of type I interferon (IFN $\alpha$ 3)

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**Abstract:** The transcription factor interferon regulatory factor 3 (IRF3) plays an important role in host defence against viral infections. However, its role during bacterial infection in teleosts remains unclear. In the present study, we evaluated the antibacterial effects of *Trachinotus ovatus* IRF3 (TroIRF3) and how it regulates type I interferon (IFN). Subcellular localisation experiments, overexpression, and quantitative real-time PCR (qRT-PCR) were performed to examine the nuclear localisation signal (NLS) of TroIRF3 and its role in the antibacterial regulatory function of TroIRF3. We assessed the binding activity of TroIRF3 to the IFN $\alpha$ 3 promoter by luciferase reporter assay. The results showed that TroIRF3 was constitutively expressed at high levels in the gill and liver. TroIRF3 was significantly upregulated and transferred from the cytoplasm to the nucleus after *Vibrio harveyi* infection. *V. harveyi* infection activates TLR-mediated signaling pathways (TLR2, TLR5M, TLR5S, and TLR9), leading to the upregulation of IRF3. By overexpressing TroIRF3, the fish were able to inhibit the replication of *V. harveyi*, whereas knocking it down increased bacterial replication. Moreover, the overexpression of TroIRF3 increased type I interferon (IFN $\alpha$ 3) production and the antibacterial-related genes (including IL11, IL15, and IL34). The NLS, which is from the 64–127 amino acids of TroIRF3, contains the basic amino acids KR74/75 and RK82/84. The results proved that NLS is required for the efficient nuclear import of TroIRF3 and that the NLS domain of TroIRF3 consists of the key amino acids KR74/75 and RK82/84. The findings also showed that NLS plays a key role in the antibacterial immunity and upregulation of TroIFN $\alpha$ 3 induced by TroIRF3. Moreover, TroIRF3 induces TroIFN $\alpha$ 3 promoter activity, whereas these effects are inhibited when the NLS domain is deficient. Overall, our results suggested that TroIRF3 is involved in the antibacterial immunity and regulation of type I IFN in *T. ovatus* and that the NLS of TroIRF3 is vital for IRF3-mediated antibacterial responses, which will aid in understanding the immune role of fish IRF3.

## Molecular Characterization And Expression Analysis Of B-cell Lymphoma-2 In *Trachinotus ovatus* (TroBcl2) And Its Role In Apoptotic Process

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**Abstract:** B-cell lymphoma- 2 (Bcl-2) is the first identified member of Bcl-2 family that exerts the anti-apoptotic function in mammals. However, its role in teleost is not fully understood. In this study, Bcl-2 of *Trachinotus ovatus* (TroBcl2) was cloned and its role in the process of apoptosis was also investigated. The complete coding region length of TroBcl2 is 687 bp encoding 228 amino acids. Four conserved Bcl-2 homology (BH) regions and one invariant “NWGR” motif located in BH1 were identified in TroBcl2. In healthy *T. ovatus*, TroBcl2 was widely distributed in eleven tested tissues and higher expression levels were shown in immune related tissues, such as spleen and head kidney. After stimulation with lipopolysaccharide (LPS), the expression of TroBcl2 in head kidney, spleen, and liver were significantly upregulated. In addition, TroBcl2 was found to be localized in both cytoplasm and nucleus by subcellular localization analysis. Functional experiments showed that TroBcl2 could enhance the cell vitality and inhibit apoptosis, possibly via reducing the mitochondrial membrane potential loss and decreasing DNA fragmentation. Moreover, upon LPS stimulation, overexpression of TroBcl2 suppressed the activation of several apoptosis related genes, such as *BOK*, *caspase-9*, *caspase-7*, *caspase-3*, *cytochrome c*, and *p53*. Meanwhile, knockdown of TroBcl2 significantly increased the expression of those apoptosis related genes. Besides, TroBcl2 overexpressed or knockdown could induce or inhibit the transcription of NF- $\kappa$ B and regulate the expression of genes (such as *NF- $\kappa$ B1*, *c-Rel*) in NF- $\kappa$ B signaling pathway and downstream inflammatory cytokines *IL-1 $\beta$* . Overall, our study suggested that TroBcl2 exerts the conserved anti-apoptotic function by mitochondrial pathway and it may serve as an anti-apoptotic regulator in *T. ovatus*.

**Keywords:** *Trachinotus ovatus*; Bcl-2; Mitochondria; Apoptosis; NF- $\kappa$ B

## Genetic Mutations in *jamb*, *jamc*, and *myomaker* Revealed Different Roles on Myoblast Fusion and Muscle Growth

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**Abstract:** Myoblast fusion is a vital step for skeletal muscle development, growth, and regeneration. Loss of *Jamb*, *Jamc*, or *Myomaker* (*Mymk*) function impaired myoblast fusion in zebrafish embryos. In addition, *mymk* mutation hampered fish muscle growth. However, the effect of *Jamb* and *Jamc* deficiency on fish muscle growth is not clear. Moreover, whether *jamb;jamc* and *jamb;mymk* double mutations have stronger effects on myoblast fusion and muscle growth remains to be investigated. Here, we characterized the muscle development and growth in *jamb*, *jamc*, and *mymk* single and double mutants in zebrafish. We found that although myoblast fusion was compromised in *jamb* and *jamc* single or *jamb;jamc* double mutants, these mutant fish showed no defect in muscle cell fusion during muscle growth. The mutant fish were able to grow into adults that were indistinguishable from the wild-type sibling. In contrast, the *jamb;mymk* double mutants exhibited a stronger muscle phenotype compared to the *jamb* and *jamc* single and double mutants. The *jamb;mymk* double mutant showed reduced growth and partial lethality, similar to a *mymk* single mutant. Single fiber analysis of adult skeletal myofibers revealed that *jamb*, *jamc*, or *jamb;jamc* mutants contained mainly multinucleated myofibers, whereas *jamb;mymk* double mutants contained mostly mononucleated fibers. Significant intramuscular adipocyte infiltration was found in skeletal muscles of the *jamb;mymk* mutant. Collectively, these studies demonstrate that although *Jamb*, *Jamc*, and *Mymk* are all involved in myoblast fusion during early myogenesis, they have distinct roles in myoblast fusion during muscle growth. While *Mymk* is essential for myoblast fusion during both muscle development and growth, *Jamb* and *Jamc* are dispensable for myoblast fusion during muscle growth.

**Keywords:** Myoblast fusion; Muscle growth; *jamb*; *jamc*; *myomaker*

# Revealing the Poor Use of Dietary Carbohydrate Phenotype in Rainbow Trout *Oncorhynchus mykiss*: Its Epigenetic Consequences and Metabolic Modulation through a Programming Strategy

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**Abstract:** The carnivorous rainbow trout (*Oncorhynchus mykiss*) is considered as a poor user of dietary carbohydrates. Recent studies showed that a high-carbohydrate/low protein diet inducing hepatic global DNA hypomethylation could be involved in the establishment/maintenance of the poor dietary carbohydrate utilization phenotype in trout, but the detail mechanisms remain unclear. The present study aimed at investigating the epigenetic mechanisms underlying this poor dietary carbohydrate utilization phenotype in trout, and exploring if the glucose metabolism and the epigenome in juveniles can be programmed through a hypoxic stimulus during early life. We first investigated the causes, processes and potential mechanisms about the hepatic global DNA hypomethylation in trout after feeding a high carbohydrate/low protein diet. Results for the first time demonstrated that a decrease in protein content and an increase in carbohydrate content in the diet can independently induce hepatic global DNA hypomethylation in trout. This global loss of methylation could be established through an active demethylation pathway and probably occurred at non-C<sup>m</sup>CGG sites. We also found that a strong hyperglycemia induced by glucose injection can also elicit a reduction in hepatic 5-mdC content and global C<sup>m</sup>CGG hypomethylation in trout. The detailed mechanisms of these demethylation processes remain to be elucidated. Besides, through metabolic programming strategy, we confirmed for the first time that using a non-nutritional stimulus, hypoxia, during early life stage persistently modulates the transcription of glucose metabolism-related genes in juvenile trout without negative effects on growth performance. Moreover, acute and chronic hypoxia tended to induce opposite programming effects on glucose-transporter encoding genes in both liver and muscle of juvenile trout. Together, the present study brings forward our understandings about the roles of epigenetics in contributing to the insufficient utilization of dietary carbohydrates in trout, and sheds light on the potential of using hypoxia as the stimulus in metabolic programming strategy to tailor the glucose metabolism, the epigenome and dietary carbohydrate utilization in rainbow trout.

**Keywords:** Nutritional programming, 5-Methylcytosine, Hypomethylation, Glucose, Fish

## Shell color polymorphism and metabolic activity in hatchery-reared juveniles of silver-lip pearl oyster, *Pinctada maxima*

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**Abstract:** Color polymorphism, where alternative color morphs occur within a single species, is a common characteristic in natural populations, and is of particular interest because, if under genetic control, this visible variation provides a way to understand genetic adaptations within a population. Shell colors have been already successfully used as makers for selective breeding in molluscs, and in the particular case of pearl oysters, the shell color plays a major role in the quality and color of pearls during pearl production process. The silver-lip pearl oyster, *P. maxima*, naturally distributed in southeast Asian countries the Western Pacific coast of Australia, is famous for producing large-sized pearls and has highly value in the pearl production industry. The shell color polymorphism and its correspondent metabolic activities in hatchery-reared *Pinctada maxima* juveniles were described in the present study. Analysis of color distances assessed the similarities among the different phenotypes (Ph); from the most common Ph-1 (grey), Ph-2 (bronze), Ph-3 (white), Ph-4 (brown), Ph-5 (green), Ph-6 (purple), and Ph-7 (yellow). Yellow colored shells (Ph-7) were found less unrelated to the other colored shells and also exhibited higher enzymatic activity, particularly those enzymes that have been previously linked to yellow or golden shell color in different bivalves. By contrast, green colored shells (Ph-5) exhibited comparatively higher levels of enzymes known to promote fast growth in various organisms, which is consistent considering that green colored shells also exhibited largest biometric values. These findings open the possibility to use shell color as a reliable indicator to breed high-quality pearl oysters with fast growth rate. These results open the possibility to use shell color as a reliable indicator to breed high-quality pearl oysters with fast growth rate and examine a potential approach to predict the colors of pearls produced by *P. maxima*.

**Keywords:** *Pinctada maxima*; shell color; polymorphism; metabolic activity

## Byssogenesis and Shell Morphology Relationships in Cultivated Winged Pearl Oyster from Hainan, P. R. China

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**Abstract:** The widely cultivated winged pearl oyster *Pteria penguin* is renowned for producing half pearls, known as mabé. Efforts for free round pearl production have faced challenges as oysters quickly reject most inserted nuclei, attributed to their strong and rigid byssus for substrate attachment. The byssus' proximity to the developing pearl-sac has potential implications for both sac formation and rounded pearl quality. This underscores the critical need for an accurate description of byssogenesis in *P. penguin*. Therefore, this research examined into the interrelationships between byssus growth dynamics, shell morphological traits, and the byssus developmental patterns over time in cultivated juveniles and adults of the winged pearl oyster. In juvenile oysters, byssus growth and its association with shell attributes were explored through monthly or bimonthly collections over a year. Notably, a diminishing trend in byssus diameter (BD) was observed as oysters aged, with growth plateauing after day 257. Furthermore, the BD to total wet weight (TW) ratio exhibited a steady decline, indicating a correlation between byssus diameter and oyster weight. Initially insignificant, the relationship between BD and shell traits became more pronounced as the oysters grew, emphasizing the importance of oyster size and weight in this correlation. By day 183, oysters exhibited higher  $r^2$  values, signifying a stronger association between BD and shell traits compared to the 366-day cultivation period. Concurrently, the occurrence of oysters without byssus decreased from 30% initially to 0% after a year of cultivation, indicating reduced susceptibility to byssus shedding as oysters matured. Conversely, in older oysters (18- and 30-month-old groups), relationships between shell morphological traits and dimensions were scrutinized. Positive and significant correlations were found between shell height (SH) and various dimensional traits, with allometric growth evident in both groups. The total wet weight to shell height ratio exhibited a clearer tendency to increase relative to exterior dimensions in older oysters, suggesting weight heterogeneity in younger oysters. These findings emphasize the significance of considering oyster weight and size in pearl cultivation, as they can indirectly affect the final pearl product's quality. Understanding the intricate dynamics between byssus growth, shell traits, and oyster age provides valuable insights for optimal pearl production strategies and oyster selection.

**Keywords:** winged pearl oyster; byssus growth; morphological traits

## Effects of Salinity Stress on the Growth Performance, Health Status, and Intestinal Microbiota of Juvenile *Micropterus salmoides*

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**Abstract:** Soil salinization has become an increasingly serious problem due to the continuous deterioration of the global environment. A large amount of land is unused due to salinization, and this represents a large waste of resources. *Micropterus salmoides* is a freshwater fish that has become increasingly popular among consumers in recent years. We investigated the effects of salinity stress on the growth performance, health status, and intestinal microbiota of juvenile *M. salmoides*. More than 480 juveniles were reared in tanks at salinity levels of 0, 5‰, 10‰, and 15‰ for 6 weeks. The weight gain rate and specific growth rate significantly decreased as salinity increased. Superoxide dismutase (SOD) activity significantly increased in the 5‰ salinity group. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP), alkaline phosphatase (AKP) activity and total antioxidant capacity (T-AOC) significantly increased when the salinity was 15‰ ( $P < 0.05$ ). Catalase (CAT), and glutathione peroxidase (GSH-PX) activity was highest in the control group. This indicates that 5‰ salinity can enhance the immune function of *M. salmoides*. However, excessive salinity can damage the immune system of *M. salmoides*. Paraffin sections of intestinal tissue revealed that villus length decreased and the magnitude of epithelial cell expansion increased as salinity increased. According to intestinal microbiota analysis, the dominant intestinal microbial taxa in *M. salmoides* were altered in saline environments. The abundance of *Bacillus* was significantly increased at a salinity of 10‰. Clusters of Orthologous Groups enrichment analysis revealed that salinity stress mainly affected lipid metabolism and amino acid metabolism. We also identified an important pathway called replication recombination and repair that was significantly enriched in the 15‰ salinity group. This indicates that the normal life activities of *M. salmoides* were maintained under a saline environment via the regulation of amino acid metabolism and lipid metabolism, as well as the salinity stress response mechanism. In sum, *M. salmoides* can survive normally at a salinity less than 5‰. It could thus be used as a saline–alkaline culture species. The results of our experiment provide new insights into the salinity tolerance of freshwater fish and will enhance the use of saline–alkaline land.

**Keywords:** *Micropterus salmoides*; salinity stress; growth; health status; intestinal

## Isolation, Identification, Drug Susceptibility Test and Pathogenicity Analysis of $\gamma$ -hemolytic *Streptococcus iniae* from *Selenotoca multifasciata*

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**Abstract:** *Streptococcus iniae* is a fish pathogenic bacterium distributed worldwide. It has a wide range of hosts, strong infectivity, and high mortality, which severely threatens the healthy development of the aquaculture industry. In a farm in Lingshui County, Hainan Province, China, a disease broke out in November 2022 in *Selenotoca multifasciata* showed spinning swimming, bleeding from fins and abdominal skin, congestion in the internal organs and brain and caused many deaths. A dominant strain named HN-1 was isolated from the brain of the dying *S. multifasciata* in this study. Artificial immersion infection experiment showed that the 7days- $LD_{50}$  of HN-1 to *S. multifasciata* was  $7.6 \times 10^3$  CFU·mL<sup>-1</sup> and similar symptom of the natural case was observed. HN-1 was identified as serotype I  $\gamma$ -hemolytic *S. iniae* according to the morphological observation, physiology characteristics, biochemical identification, 16S rRNA and *lctO* gene sequence analysis and serotype analysis. Drug susceptibility test showed that the *S. iniae* HN-1 was sensitive to 19 drugs such as florfenicol, doxycycline, ciprofloxacin, penicillin, kanamycin, meropenem, bactrim and furazolidone, resistant to gentamycin, neomycin, roxithromycin, erythromycin, lincomycin, clindamycin. Seven virulence genes including *scpI*, *simA*, *pdi*, *sagA*, *cpsD*, *pgmA* and *cfi* were detected in HN-1 using PCR technology. Comparing to those in  $\beta$ -hemolytic *S. iniae* TO-1, the mRNA expressions of *scpI*, *simA*, *pdi*, *pgmA*, and *cfi* in HN-1 were significantly up-regulated ( $P < 0.05$ ), while those of the streptolysin S related genes including *sagA*, *sagB*, *sagC*, *sagD*, *sagE*, *sagF*, *sagG*, *sagH*, and *sagI* were significantly down-regulated ( $P < 0.05$ ). HN-1 might have distinct mechanisms in adhesion, colonization, invasion and immune evasion. The results will provide a reference basis for the prevention and treatment of *S. iniae*.

**Keywords:** *Selenotoca multifasciata*; *Streptococcus iniae*;  $\gamma$ -hemolytic; drug susceptibility test; virulence genes

## Comprehensive mRNA and MicroRNA Analysis Revealed the Effect and Response Strategy of Grass Carp Under Geosmin Exposure

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**Abstract:** Geosmin is an environmental pollutant that causes off-flavor in water and aquatic products. The high occurrence of geosmin contamination in aquatic systems and aquaculture raises public awareness, however, few studies have investigated the response pathways of geosmin stress on freshwater fish. In the current research, grass carp was divided into control and exposure groups (50 $\mu$ g/L geosmin). After 96h of exposure, liver tissue was sequenced and validated using real-time quantitative polymerase chain reaction (qPCR). The results of mRNA sequencing showed that there are 528 genes up-regulated and 488 genes down-regulated. DNA-adducts pathway was enriched which suggests that geosmin present genotoxic to grass carp. Members of cytochrome P450 and uridine diphosphate (UDP)-glucuronosyltransferase were up-regulated. Two known microRNAs were up regulated and 15 were down regulated. KEGG analysis of differential expression genes showed that chemical carcinogenesis-DNA adducts, metabolism of xenobiotics by cytochrome P450, drug metabolism-cytochrome P450 pathway was enriched. Common genes from the target genes of microRNAs and differential expression genes enriched in metabolism of xenobiotics cytochrome P450 pathway. Network of miRNA-mRNA showed that there are 70 positive and 42 negative miRNA-mRNA interactions. Two miRNAs (dre-miR-146a and miR-212-3p) down regulated their target genes (*LOC127510138* and *adh5*, respectively) which are enriched cytochrome P450 related pathway. The results show that geosmin present genotoxic to grass carp. The data also indicated that cytochrome P450 system and UDP-glucuronosyltransferase play essential roles in biotransformation of geosmin. MicroRNA (dre-miR-146a and miR-212-3p) involved in biotransformation of geosmin via downregulating their target genes. This study provided novel insights into the response of geosmin exposure and contribute to the development of strategies for managing its negative impacts in both natural and artificial environments.

**Keywords:** Geosmin; Transcriptomic; microRNA sequencing; Cytochrome P450; UDP-glucuronosyltransferase

## Impact of *Microcystis aeruginosa* on Hepatotoxicity, Metabolic Effect and Liver Transcriptome Analysis of Japanese Eel (*Anguilla japonica*)

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**Abstract:** Microcystins (MCs) are the hepatotoxins produced during blooms of cyanobacterial cells released into the column of water. *Microcystis aeruginosa* is a large-scale, widespread toxin-producing algal that produces MCs in lakes and ponds worldwide. This study investigated the effect of *M. aeruginosa* toxins on Japanese eel (*Anguilla japonica*) pathological damage, metabolism and uncovered the mechanism through metabolic transcriptome, after chronic exposure. Fish were exposed to 50 µg kg<sup>-1</sup> body weight per day MC-LR contained in *M. aeruginosa* cells. 7, 14, and 21 days after exposure, the blood serum was sampled for biochemical analysis, the liver was also sampled for transcription analysis, and part of the liver, gills, and intestine was sampled for histological analysis. The activity of Alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine transaminase (ALT) activities were significantly higher in the treated fish, but oxidative stress enzyme superoxide dismutase (SOD), and chloramphenicol acetyltransferase (CAT) activities were significantly lower ( $P < 0.05$ ). Histological damages were also observed in treating fish organs. The changes of lipid metabolism genes that ACACA, ASAH2, and PISD mRNA expression were significantly up-regulated ( $P < 0.05$ ), and the MCs exposure caused no change in ELOVL6 and TER mRNA expression ( $P > 0.05$ ). All results demonstrated that *M. aeruginosa* could negatively impact the hepatic functions in Japanese eel.

**Keywords:** MC-LR; Cyanobacterial harmful algal blooms; toxicity; Metabolic effect; RNA-Sequence; *Anguilla japonica*

## Male-biased Sexual Size Dimorphism in Captive Largemouth Bass

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**Abstract:** Sexual size dimorphism (SSD) is a prevalent characteristic in aquatic animals, with variations in size between males and females driven by numerous external and internal factors. However, conflicting evidence and emerging findings surrounding SSD in largemouth bass *Micropterus salmoides* (LMB) have generated controversy. To elucidate the potential sexual bias and investigate the underlying mechanisms, we conducted a systematic sampling of captive LMB from 15 DPH to 360 DPH. Our findings indicated that growth displayed positive allometry in females ( $W = 0.00332 \times TL^{3.439}$ ) and negative allometry in males ( $W = 0.0144 \times TL^{2.994}$ ), and incompletely conformed to the Rensch's rule. Males significantly surpassed females in size from 240 DPH and the body weight of males ( $384.10 \pm 13.53$  g) was significantly higher than the females ( $328.54 \pm 15.48$  g) by 16.91% at 360 DPH. Further analysis of the volume percentage of different stages of oocytes provides a base for excellent regression between the volume percentage of the primary growth stage, cortical alveoli stage, vitellogenesis stage and gonadal development. At the early stage when the growth of female fish was slower than the males, it was observed that the volume of oocytes in cortical alveoli stage and vitellogenesis stage increased significantly in the ovaries. The ratio of 11-KT /  $17\beta$ -E<sub>2</sub> of male fish was highest at 150 DPH, and the ratio of  $17\beta$ -E<sub>2</sub> / 11-KT of female fish was highest at 180 DPH, which indicated that the SSD was closely related to the gonad development of male and female fish. We speculated that the large energy consumption for ovarian development was the main reason for the SSD. The findings of this work provide theoretical basis for the relationship between SSD and gonad development, and solution for problems associated with gonadal development.

**Keywords:** Largemouth bass; Sexual size dimorphism; Gonadal development;  $17\beta$ -Estradiol; 11-keto Testosterone

## Tilapia Cage Culture: An Imperative Approach for Better-Off Resilient Livelihood to Adverse Climatic Changes and Natural Disasters in Coastal Bangladesh

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**Abstract:** Globally, fish farming in cages is a promising aquaculture technology and currently, one of the best prospects for commercial aquaculture in Europe and many Asian countries. But in Bangladesh, cage aquaculture is yet to be widely practiced. However, Bangladesh is well-endowed with diversified fisheries resources comprising rivers, tributaries, haors, oxbow lakes and wetlands etc. With access to these resources, cage culture as an alternative livelihood is gaining popularity among resource-poor fishing communities for income, nutrition and women empowerment. However, a participatory experiment of cage cultures of Nile tilapia (*Oreochromis niloticus* L.) with impoverished women-led households was carried out from June to November 2019 and simply evaluated the production and economics of cage cultures, income and nutrition of households, gender roles and challenges. A total of twenty-seven nylon net cages (5.5 m × 3.5 m × 1.5 m; 1.0 cm mesh) were installed in community brackish water ponds in three saline-prone coastal districts under 3 treatments (N= 27; n= 9 at each site). T<sub>1</sub> was the controlled-cage culture of Nile tilapia; T<sub>2</sub>-cage culture of Nile tilapia, where tilapia are sold out to markets; and T<sub>3</sub>-cage culture of Nile tilapia, where tilapia are consumed at households. After 45 days of culture, T<sub>2</sub> and T<sub>3</sub> started harvesting weekly with 20 larger fish. All cages were stocked with larger-sized tilapia fingerlings (avg. 34.16±1.22 g) at an optimum unique density of 45 fish/m<sup>3</sup> (1260 fish/cage). The fish were hand-fed with commercial floating feed at 8-4% body weight twice daily, with 60% at ~9.00 am and 40% at ~3.00 pm for 90 days each in two culture cycles.

Almost a similar survival rate (%) was recorded in all treatments, ranging from 95.63±0.87% to 97.17±1.22%. The mean final weights of tilapia were 278.56±14.37 g, 323.19±11.24 g and 321.06±13.50 g, and the gross productions of tilapia were 335.65±11.10 kg/cage, 386.48±7.69 kg/cage and 384.55±8.22 kg/cage in T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively, indicating significantly higher mean final weights and gross productions in T<sub>2</sub> and T<sub>3</sub> than T<sub>1</sub> (Table 1). The specific growth rates and FCR were significantly higher (p < 0.05) in T<sub>2</sub> and T<sub>3</sub> than T<sub>1</sub>, but T<sub>2</sub> and T<sub>3</sub> showed no significant differences (Table 1). Again, significantly higher (p < 0.05) net revenue was produced by T<sub>2</sub> and T<sub>3</sub> than T<sub>1</sub>. After the interventions, the tilapia consumption in terms of meals (no./7 days) and quantity (g/7 days) has been increased significantly (p < 0.001) by 29.34% (3.17±0.74 to 4.10±0.69) and 42.38% (0.538±0.181 g to 0.766±0.148 g) respectively in T<sub>3</sub> households. Similarly, the average monthly income in T<sub>2</sub> households has

increased by 27.19% during partial harvesting and 50.73% in final harvests overall in all treatments. Women were at the forefront of this trial and had access to family finance, but access to common fisheries resources is limited by existing policies and power exercises. Salinity (5-10 ppt) and temperature (26-32<sup>o</sup> C) had no adverse effects on production. Thus, cage culture is prolific and holds immense potential for boosting aquaculture in Bangladesh.

**Keywords:** Cage culture; Tilapia; Income and nutrition; Women empowerment; Climate change

Table 1. Growth and production performances of tilapia cage cultures in three treatments (mean  $\pm$  SD)

Treat ment	Initial Weight (g)	Final Weight (g)	SGR (%/day)	FCR	Survival Rate (%)	Fish Consumed & Sold Out (no.; kg/cage/45 days)	Gross Production kg/cage/90 days
T1	33.51 $\pm$ 0.91	278.56 $\pm$ 14.37 <sup>b</sup>	2.35 $\pm$ 0.2 <sup>b</sup>	1.233 $\pm$ 0.4 <sup>a</sup>	95.63 $\pm$ 0.87	-	335.65 $\pm$ 11.10 <sup>b</sup>
T2	34.03 $\pm$ 1.13	323.19 $\pm$ 11.24 <sup>a</sup>	2.50 $\pm$ 0.1 <sup>a</sup>	1.072 $\pm$ 0.2 <sup>b</sup>	96.87 $\pm$ 1.56	140 P; 35.84 $\pm$ 4.67	386.48 $\pm$ 7.69 <sup>a</sup>
T3	34.31 $\pm$ 0.67	321.06 $\pm$ 13.50 <sup>a</sup>	2.48 $\pm$ 0.1 <sup>a</sup>	1.078 $\pm$ 0.0 <sup>b</sup>	97.17 $\pm$ 1.22	140 P; 35.14 $\pm$ 5.37	384.55 $\pm$ 8.22 <sup>a</sup>

## Distribution Patterns of Glucocorticoid Receptor Genes in The Brain of Yellowtail Clownfish *Amphiprion clarkii*

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**Abstract:** Glucocorticoids (GCs) are stress hormones found in the hypothalamus-pituitary-adrenal axis (HPA axis) of vertebrates. They regulate various physiological functions, including metabolism, growth and development, immunity, inflammation, and reproductive physiology, through the glucocorticoid receptor (GR). The yellowtail clownfish (*Amphiprion clarkii*) is a protandrous hermaphrodite fish, whose sex differentiation and gonad development are closely related to its social status. To investigate the potential physiological roles of glucocorticoids and GRs, our study employed a novel RNAscope technique to examine the distribution patterns of *gr1* and *gr2* gene in the brain of yellowtail clownfish. The results indicated that *gr1* and *gr2* mRNA positive signals were significantly expressed in various brain regions, including medial dorsal telencephalic area (Dm3, Dm2, Dlp, Dp), postcommissural part of the ventral telencephalon (Vp), preoptic area (NPOpc, NPOav, PMgc), anterior periventricular nucleus (NAPv), optic tectum (OT), dorsal part of the nucleus of the lateral recess; (NRLd), ventral part of the lateral tuberal nucleus (NLTv), nucleus of the posterior recess (NRP), inferior part of the lateral tuberal nucleus (NLTI), and lateral part of the diffuse nucleus of the inferior lobe (NDLII). In contrast, corpus of the cerebellum (CCe) and periventricular nucleus of the posterior tuberculum (TPp) displayed lower expression. Moreover, *gr2* mRNA positive signals exhibited high expression in regions such as the habenular nucleus (NH), tegmentum (TEG), posterior part of glomerular nucleus (NGp), nucleus preglomerulosum pars medialis commissularis (NPGc), posterior tuberal nucleus (NPT), and nucleus of the medial longitudinal fasciculus (nMLF), while *gr1* showed lower expression. These findings suggest that glucocorticoids, mediated by GR, are involved in various physiological functions, including emotional memory, spatial learning, visual motor function, stress regulation, and reproductive regulation in different brain regions of yellowtail clownfish. This study further underscores the highly conserved expression and function of GR in vertebrates, providing a theoretical foundation for understanding the regulatory role of glucocorticoids and their receptors in the central nervous system.

**Keywords:** glucocorticoid receptor; *Amphiprion clarkii*; RNAscope

## A Comparative Study on the Growth Characteristics of juvenile pearl oyster *Pinctada martensii* at Different water depths

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**Abstract:** To optimize the cultivation management techniques of *Pinctada martensii*, different water depths (3m, 5m, and 7m) were choose to tests for *P. martensii* juvenile farming, and the survival rate and growth parameters (wet weight, shell length, shell width, and shell height) were compared. The cultivation testing period spanned six months (November 2022 to May 2023). The survival rates and growth performance were recorded every month. A significant decline in survival rates one month after cultivation, which then trended to stabilized. After first month of cultivation, the survival rate was highest at a depth of 3m ( $89 \pm 1\%$ ), while the survival rate of *P. martensii* at 7 m depth was the lowest ( $81 \pm 5\%$ ). Furthermore, the survival rates for depths of 3 m, 5 m, and 7 m after the six-month trial were  $84.12 \pm 3.10\%$ 、 $80.23 \pm 1.43\%$ , and  $64.72 \pm 2.11\%$ , respectively after six months of cultivation. The growth parameters (wet weight, shell length, shell width, and shell height) showed a relatively consistent increasing trend. From December 2022 to February 2023, there was a slow upward trend in wet weight, shell length, shell width, and shell height. While a rapid and stable growth trend was observed from February 2023 to April 2023. However, the different growth trends appeared among the groups in May 2023, where the 3 m and 5 m group maintaining a rapid growth trend, while the 7 m group tended towards stability. After the six-month cultivation the wet weights at depths of 3 m, 5 m, and 7 m were  $15.36 \pm 1.12$  g、 $14.58 \pm 0.70$  g, and  $10.16 \pm 1.77$  g ( $P < 0.05$ ) respectively; and the corresponding shell lengths were  $42.92 \pm 1.74$  mm、 $41.73 \pm 2.12$  mm, and  $37.19 \pm 1.75$  mm ( $P < 0.05$ ); and the corresponding shell widths were  $41.73 \pm 0.47$  mm、 $40.61 \pm 0.85$  mm, and  $36.96 \pm 1.37$  mm ( $P < 0.05$ ); and the corresponding shell heights were  $15.29 \pm 0.49$  mm、 $15.29 \pm 0.53$  mm, and  $13.73 \pm 0.13$  mm ( $P < 0.05$ ). In conclusion, it can be concluded that depths ranging from 3m to 5m are more conducive to the growth of *P. martensii* juvenile compared to the 7m depth.

**Keywords:** *Pinctada martensii*; juvenile; water depth; survival rate; growth performance

## Recombinase Polymerase Amplification assay for rapid Detection of *Cryptocaryon irritans*

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**Abstract:** *Cryptocaryon irritans* is a holotrichous ciliate that parasites marine fishes in temperate and tropical seas, causes marine white spot disease, and can infect almost all marine teleost fish, it has caused massive deaths and serious economic losses. So it's particularly important and urgent to study early rapid detection technology. The isothermal amplification technology recombinase polymerase amplification (RPA) is an alternative molecular technique that has been successfully used for field diagnosis of various pathogens. The technique has been widely used recently due to its affordable price, high sensitivity, short reaction time and simplicity and has been used in a suitcase laboratory. The study aimed to realize the rapid and accurate detection of *Cryptocaryon irritans* in the field, the specific primers were designed according to the conserved region of the ribosome 18SrRNA fragment of *Cryptocaryon irritans* by using the recombinant enzyme polymerase isothermal amplification, and the RPA detection method of *Cryptocaryon irritans* was established. The RPA reaction was performed at a constant temperature of 39°C for 30 min. The results showed that the RPA detection method was highly specific for *Cryptocaryon indicum* stimulated by Golden pomfret, the specific target fragment of 232 bp was amplified. The sensitivity of the *Cryptosporidium irritans* RPA detection method can reach 4 *Cryptozoon irritant* worms in the sample. This method has high amplification efficiency, short detection time, good specificity and high sensitivity, and is suitable for rapid detection of *Cryptocaryon irritans*.

**Keywords:** *Cryptocaryon irritans*; recombinase polymerase amplification; rapid detection

## Screening and Application of Phytochemicals for Inhibiting *Vibrio harveyi* Biofilm

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**Abstract:** *Vibrio harveyi* is a gram-negative marine luminescent bacterium, which has the characteristics of high mortality and wide host range, causes huge economic losses to aquaculture industry. Biofilm is a key factor for *V. harveyi* to survive in water. Biofilm can protect bacteria against external dangers and enhance their resistance to antimicrobial agents, which plays an important role in the pathogenicity of *V. harveyi*. The incidence of biofilm mediated antibiotic resistance stresses the quest for novel therapeutics to treat vibriosis in aquaculture. The aim of this paper is to screen phytochemicals as novel attenuating virulence agents of *V. harveyi* by inhibiting biofilm formations. The formation of *V. harveyi* biofilm on microwell plates was observed and quantified by crystal violet staining. The effects of 30 phytochemicals on the biofilm formation of *V. harveyi* at 10 µg/mL was investigated. Then, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of this phytochemical were determined by broth dilution method. The effects of the phytochemical on the established biofilms and the motility ability (swarming and swimming) of the *V. harveyi* were measured at sub-inhibitory concentrations using crystal violet staining method and semi-solid culture method; Results showed that the inhibitory rates of fisetin, celastrol, gastrodin, piperine, esculin, acteoside and hispidulin were 75.6%, 39%, 43.5%, 51.5%, 33.5%, 36.5%, and 55.5%, respectively at 10 µg/mL. The MIC and MBC of fisetin on *V. harveyi* were 128 µg/mL and 256 µg/mL, respectively. Fisetin under sub-inhibitory concentration not only reduced the formation of *V. harveyi* biofilm, but also destroyed the established biofilm in a dose-dependent manner. Fisetin reduced the swimming and swarming motility of *V. harveyi* in a dose-dependent manner. Collectly, our findings suggested that fisetin might be a promising phytochemical for attenuating *V. harveyi* biofilm formation and virulence.

**Keywords:** *Vibrio harveyi*; Phytochemicals; Biofilm

## Screening and Application of Natural Inhibitors of *Streptococcus agalactiae* Virulence

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**Abstract:** *Streptococcus agalactiae* is one of the most predominant pathogens in aquaculture. The morbidity and mortality of the streptococcosis caused by *S. agalactiae* in tilapia is respectively higher than 20%-50% and 60%.-80%. It is an urgent issue for prevention and treating the streptococcosis. Comparing to conventional antibiotics, anti-virulence therapeutic strategies not only fight infections but also have low probability of causing drug resistance due to low survival pressure caused by the lack of virulence factors. Beta-hemolytic/cytolytic ( $\beta$ -h/c) and biofilm are the key virulence factors of *S. agalactiae*. In the present study, the effects of 40 natural compounds at 10 $\mu$ g/mL on *S. agalactiae* PBSA0901  $\beta$ -h/c activity and biofilm were determined by the microtiter plate method and the crystal violet staining method, respectively. And then the natural compounds with the highest inhibitory effects  $\beta$ -h/c` were screened out and their half maximum inhibitory concentrations ( $IC_{50s}$ ) were further determined. The natural compound with the lowest  $IC_{50s}$  on  $\beta$ -h/c and biofilm was screened out and its effects on the susceptibility of *S. agalactiae* PBSA0901 to lysozyme and hydrogen peroxide ( $H_2O_2$ ) were further determined. Results showed that  $\beta$ -h/c activity-inhibition ratios of 10  $\mu$ g/mL 19 natural compounds were higher than 10% and biofilm-inhibition ratios of 10  $\mu$ g/mL 17 natural compounds were higher than 10%. The  $\beta$ -h/c activity-inhibition ratios of xanthohumol, isoxanthohumol, licochalcone A, corosolic acid, and celastrol reached 100%. Their  $IC_{50s}$  on  $\beta$ -h/c activity of were  $2.926 \pm 0.13$ ,  $9.039 \pm 0.27$ ,  $1.356 \pm 0.018$   $\mu$ g/mL,  $4.876 \pm 0.082$   $\mu$ g/mL, and  $4.351 \pm 0.37$   $\mu$ g/mL, respectively. The biofilm-inhibition ratios of 10  $\mu$ g/mL xanthohumol, fisetin, paeonol, thymol, corosolic acid, and isoxanthohumol were higher than 60% and the  $IC_{50s}$  of xanthohumol, isoxanthohumol, and corosolic acid biofilm were  $31.673 \pm 0.21$ ,  $62.001 \pm 0.41$ , and  $42.007 \pm 0.31$   $\mu$ g/mL, respectively. Xanthohumol with the lowest  $IC_{50s}$  on  $\beta$ -h/c and biofilm was screened out. Xanthohumol at 6.25  $\mu$ g/mL significantly increased the sensitivity of *S. agalactiae* to  $H_2O_2$ . Xanthohumol has great potential on prevention and treating streptococcosis.

**Keywords:** *Streptococcus agalactiae*; natural compounds; biofilm; inhibitors; tilapia

## ***α-Mangostin* of Bacteriostatic Action of *Streptococcus iniae* Dolphin and Effects on Diseased *Trachinotus ovatus***

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**Abstract:** *Streptococcus iniae* is one of the most important fish pathogens in the world, which seriously endangers the development of aquaculture. Natural compounds are widely used in the prevention and control of aquaculture diseases because of their advantages of low pollution, less residue and less resistance. *α-Mangostin* are natural compounds isolated from the husks of the plant *Garcinia L.* in the *Garcinia* family. Based on transcriptome technology, this article studies the inhibitory effect and mechanism of *α-mangostin* on *S. iniae* poris and evaluates the therapeutic effect of *α-mangostin* on oval pomfret with *S. iniae* ovis disease, its effect on fleshy flavor, and its pharmacokinetic characteristics. The main experimental method was to determine the growth and inhibitory concentration of *α-mangostin* on *S. iniae* dolphin with the help of in vitro experiments. The effects of *α-mangostin* on the cell membrane, the cell wall, and the morphology and protein of *S. iniae* dolphini were determined by conductivity, extracellular nucleic acid and protein leakage, protein concentration, MAD-MDH and SDH activity, SDS-PAGE, the PI and the FAD staining observations, the field emission scanning electron microscopy, and the transmission electron microscopy observations. The results showed that *α-mangostin* could effectively inhibit the growth of *S. iniae* poris; the lowest inhibitory concentration was 6.25 μg/mL. In addition, the protein, and the nucleic acid extravasation of bacterial fluid increased significantly, and the damage degree of *α-mangostin*, which was added to 2MIC and MIC concentrations, was significantly more destructive to *S. iniae* dolphini. The therapeutic effect of *α-mangostin* was studied by in vivo bacteriostatic experiments using *Trachinotus ovatus* as a model to study the therapeutic effect of curdin and to determine its muscle nutritional composition, volatile flavor components and pharmacokinetic characteristics to evaluate the safety of *α-mangostin* in aquaculture.

**Keywords:** *Trachinotus ovatus*; *Streptococcus iniae*; bacteriostatic mechanism; withdrawal period; Natural compounds

## Development and Application of a Dual Recombinase Polymerase Amplification (RPA) Assay for Detecting *Vibrio alginolyticus* and *Vibrio Parahaemolyticus*

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**Abstract:** *Vibrio alginolyticus* and *Vibrio parahaemolyticus* are very common pathogens in mariculture. In China, vibriosis caused by these two pathogens causes great economic losses to aquaculture industry every year. The development of a diagnostic method for early, real-time, in-situ, specific and accurate detection of *V. alginolyticus* and *V. parahemolyticus* is an effective means to block pathogen transmission and intervene in advance. Recombinase polymerase amplification (RPA), as a popular thermostatic amplification technique, can complete the in vitro amplification of nucleic acid target sequence through the interaction of recombinase, DNA polymerase and single strand DNA-binding protein with primer and target sequence. Due to its constant temperature and high efficiency, it has become an alternative to conventional PCR and can be used for the rapid detection of pathogens. In this study, A dual RPA combining lateral flow dipsticks (LFD) and Nfo probe detection assay (RPA-LFD) capable of simultaneously detecting *V. alginolyticus* and *V. parahemolyticus* was established based on the virulence regulatory factor (*Tox R*) genes of *V. alginolyticus* and *V. parahemolyticus*, and then screened primer probe and optimized the reaction system. Results showed that the best amplification temperature and time of the dual RPA- LFD method for detecting *V. alginolyticus* and *V. parahemolyticus* were 37 °C and 20 min, respectively. The sensitivity of the developed RPA-LFD method was as low as 1pg/μl genomic DNA and the results can be visualized within 5 min. The developed RPA-LFD method has good specificity, greatly shortens the detection time, and can be applied to in-situ detection. It has great potential in diseases diagnoses.

**Keywords:** *Vibrio alginolyticus*; *Vibrio parahaemolyticus*; recombinase polymerase amplification; lateral flow dipsticks; duplex detection

## Identification of Target Proteins of Honokiol Involved in *Cryptocaryon irritans* Apoptosis

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**Abstract:** *Cryptocaryon irritans* is a parasitic ciliate infecting marine teleost fish. Due to the lack of safe and effective prevention and control methods, *C. irritans* has caused serious harm to the marine aquaculture industry. Our previous work has demonstrated that honokiol was able to induce *C. irritans* apoptosis, but the mechanism remains unknown. This research aimed to identify the honokiol targets in *C. irritans*, and reveal the mechanism of honokiol for inducing *C. irritans* apoptosis. Sixty-one apoptosis-related proteins from the genome database of stimulated *C. irritans* (SRX12890364, SRX12890363) were used as candidates. And then the binding energy scores between honokiol and candidate proteins were calculated by molecular docking method. The candidate protein with the highest binding energy score was considered as honokiol target protein. *Cryptocaryon irritans* DNA was extracted with OMEGA DNA extraction kit, and used as amplified template. The primers were designed by Primer Premier 5, and synthesized by Liuhe Bada Gene Technology Co., LTD. The honokiol target protein gen amplification product was obtained by PCR. The PCR product was ligated into vector PET-32a was used for expressing and transformed into BL21 (DE3) for expression. The expression protein was purified and refolded. The drug affinity reaction target stabilization technique (DARTS) was used to evaluated the binding between honokiol and the target proteins. The results showed that among 61 apoptosis-related proteins, actin had the highest score -19.678 Kcal/mol. DARTS experiments showed the protein treated by Streptomyces protease had bands in the co-incubated group but no bands in the control group. Actin may be involved in the regulation of *C. irritans* apoptosis induced by honokiol.

**Keywords:** *Cryptocaryon irritans*; honokiol; actin; apoptosis; DARTS

## **Histological, Ultrastructural and Transcriptomic Responses of Grass Carp to the Exposure of Off Flavor Compound 2-Methylisoborneol (2-MIB)**

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**Abstract:** 2-Methylisoborneol (2-MIB) is a common and widely distributed off flavor compound in water. However, the toxic mechanisms of 2-MIB on aquatic organisms remain largely unexplored. Here, grass carp fingerlings were exposed to different concentrations (0, 5, and 20 µg/L) of 2-MIB for 96 hours. The accumulation of 2-MIB in the dorsal muscle was measured. The histological analysis, ultrastructure observations and transcriptomic sequencing were conducted on the liver tissues. 2-MIB was found to accumulate effectively in the fish muscle, with a significant increase in accumulation as the exposure concentration increased. Histological and ultrastructure observations indicated that 2-MIB caused concentration-dependent inflammatory infiltration and mitochondrial damage in the liver. Transcriptomic analysis revealed lipid metabolism disorders induced by 2-MIB exposure in grass carp. Additionally, 5 µg/L 2-MIB affects the neurodevelopment and cardiovascular system of grass carp fingerlings through extracellular matrix (ECM)-receptor interaction and focal adhesion pathway. Furthermore, several pathways related to the digestive system were significantly enriched, implying that 2-MIB may impact pancreatic secretion function, protein digestion and absorption processes. These findings provide new references for the potential toxicological mechanism of 2-MIB on aquatic animal. In addition, the results showed that the presence of off flavor not only reduced the quality of aquatic products, but also had adverse effects on the healthy aquaculture. Therefore, it is necessary to take corresponding techniques or measures to remove off flavor in the follow-up study.

**Keywords:** 2-Methylisoborneol; Lipid metabolism; Transcriptome; Oxidative stress; Mitochondrial damage

## The Role of Water Flow in Melanophore of Zebrafish

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**Abstract:** Japanese flounder (*Paralichthys olivaceus*) is a kind of coastal bony fish which has undergone metamorphosis and benthic life, and has obvious asymmetrical signs including asymmetrical body color. However, in cultured animals, melanosis is often observed without abnormal pigmentation on the blind side. Studies have shown that the melanism of the blind side is related to the contact with the bottom of the breeding pond, and the melanism often appears in the body edge of the eyeless side, the head, and the vicinity of the pectoral fin, etc. However, the breeding density of artificial halibut is very high, and these parts are not closely fitted with the bottom of the pond, so they are more likely to be exposed to water and stimulated by the current. Therefore, this study proposed that water flow can affect fish melanocytes on this basis. In this study, model biological Zebrafish (*Danio rerio*) were verified. After feeding zebrafish for 3 months at a relatively high flow rate (0.022m/s) compared with a low flow rate (0.010m/s), the number of skin melanocytes increased significantly, and the expression levels of melanocyte marker genes *kit* and *mitf* genes increased significantly. Transcriptome sequencing was performed on the skin, and KEGG enrichment analysis revealed that the 20 signaling pathways mainly involved in differential genes were as follows: Arachidonic acid metabolism, Cell adhesion molecules, Amino sugar and nucleotide sugar metabolism, "PPAR signaling pathway", "Purine metabolism", etc. Real-time fluorescence quantitative PCR detection showed that *pomc* of positive regulatory melanocytes was significantly down-regulated, while MC1R had no significant change, and the expression of MC1R endogenous inhibitory protein *asip1* gene was significantly down-regulated. At the same time, the gene expression level of Piezo1, a mechanoreceptor channel protein, was unchanged in the high-flow group, and Piezo2 was significantly decreased, while the mechanoreceptor channel protein TRPV4 gene was significantly up-regulated. These results suggest that water flow may influence the formation of melanocytes by down-regulating melanocyte negative regulator *asip1* through TRPV4.

**Keywords:** Fish body color; Melanophore; Water flow; *Asip1*; RNA-seq

## Study on the Immune Effect of *Vibrio anguillarum* Immunoenhancer on *Lateolabrax japonicus*

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**Abstract:** Immunopotentiator, as a popular feed additive for aquatic animals, has the function of activating immune active cells, enhancing the body's own immune function, and activating animal immunity. It can effectively prevent and control diseases in aquaculture, improve the quality and value of aquatic animals, and has been widely used in fish farming. Commonly used immune enhancers are divided into animal and plant derived immune enhancers, microbial derived immune enhancers, synthetic immune enhancers, nutritional factor based immune enhancers, etc. To study the immunoprotective effect of *Vibrio anguillarum* immunoenhancer on *Lateolabrax japonicus*. 2% of the feed weight was mixed with *Vibrio anguillarum* attenuated genetically engineered bacteria. Samples were taken at 0, 7, 14, and 21 days after feeding, and immunoglobulin M (IgM) and immunoglobulin G (IgG) were detected in *Lateolabrax japonicus* serum using ELISA. The experimental results showed that after 21 days of feeding, the levels of IgM and IgG in the serum of *Lateolabrax japonicus* significantly increased compared to the control group. For *Lateolabrax japonicus* fed with immune booster feed for 21 days, use  $1 \times 10^6$  CFU/ml wild *Vibrio anguillarum* was challenged. After 14 days of challenge, 5 *Lateolabrax japonicus* survived in the immune group, while all the control group died on the 13th day.

**Keywords:** immunopotentiator; *Lateolabrax japonicus*; Serum antibody; protective rate

## ***Clostridium butyricum* Culture Inclusion in High-carbohydrate Diet Improved Carbohydrate Utilization, Antioxidant Capacity and Intestinal Microbiota of Largemouth Bass *Micropterus salmoides***

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**Abstract:** A 60-day feeding trial was conducted to explore the potential regulatory effects of dietary *Clostridium butyricum* culture (CBC) supplementation in high-carbohydrate diet (HCD) on carbohydrate utilization, antioxidant capacity and intestinal microbiota of largemouth bass. Triplicate groups of largemouth bass (average weight  $35.03 \pm 0.04$  g), with a density of 28 individuals per tank, were fed low-carbohydrate diet and HCD supplemented with different concentration of CBC (0%, 0.25%, 0.50% and 1.00%). The results showed that dietary inclusion of CBC alleviated the hepatic glycogen accumulation induced by HCD intake. Additionally, the expression of hepatic *ampka1* and insulin signaling pathway related genes (*ira*, *irb*, *irs*, *p13kr1* and *akt1*) increased linearly with the supplementary level of CBC in HCD, which might be associated with the activation of glycolysis related genes (*gk*, *pfkl* and *pk*). Meanwhile, the expression of intestinal short chain fatty acid transport related genes (*ffar3* and *mct1*) was significantly increased with dietary inclusion of CBC. In addition, the hepatic antioxidant capacity of largemouth bass fed HCD was improved with dietary CBC supplementation, as evidenced by a linear decrease in MDA concentration and linear increase in T-AOC, T-SOD and CAT. Also, the expression of hepatic antioxidant enzyme related genes (*sod1*, *sod2* and *cat*) showed a linear increase with dietary CBC inclusion, which might be correlated with the elevated expression of *nrf2* and the reduced expression of *keap1*. The analysis of bacterial 16S rRNA V3–4 region indicated that dietary CBC inclusion significantly reduced the enrichment of *Firmicutes* and potential pathogenic bacteria genus *Mycoplasma*, but significantly elevated the relative abundance of *Fusobacteria* and *Cetobacterium*. In summary, dietary CBC inclusion improved carbohydrate utilization, antioxidant capacity and intestinal microbiota of largemouth bass fed HCD.

**Keywords:** *Clostridium butyricum* cultures; Largemouth bass; Insulin signaling pathway; Antioxidant capacity; Intestinal microbiota

## Transcriptomic Analysis of Natural Mortality in Artificially Bred Japanese Eel *Anguilla japonica* Larvae during Early Developmental Stages

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**Abstract:** After hatching, Japanese eel larvae enter a stage of transition from endogenous to exogenous nutrition which is often accompanied by large-scale mortality. In this study, we focused on the larvae that survived for 36 hours (Group A), 48 hours (Group B, dysplasia), and 60 hours (Group C, with large-scale mortality) after hatching. We conducted morphological observations and transcriptomic analysis. As survival time increased, the larvae exhibited shortened body length, body curvature, spinal deformity, and significantly reduced yolk sac and oil droplet diameters. Transcriptomic analysis revealed 5,381 differentially expressed genes among the different experimental groups, including 2,325 upregulated genes and 3,056 downregulated genes. KEGG enrichment analysis showed significant impacts on pathways such as cholesterol metabolism, fat digestion and absorption, and DNA replication and repair. Additionally, qPCR analysis of key lipid metabolism genes, including *LOC118234363*, *LOC118230117*, *LOC118229162*, *LOC118237125*, *LOC118228071*, and *apoc1*, exhibited significantly decreased expression levels. During the early developmental process of the larvae, the expression of lipid metabolism-related genes is significantly downregulated, while the expression of DNA replication and repair-related genes is significantly upregulated as time progresses. This study provides valuable reference data for artificial breeding of Japanese eel and early culture of larvae.

**Keywords:** Lipid metabolism; Survival rate; Japanese eel Larvae; Morphogenesis; Transcriptomics

## **Analysis of Intestinal Microflora of Japanese eel (*Anguilla japonica*) in Migration Along China Offshore**

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**Abstract:** In order to study the structure and diversity of the intestinal microbiota of glass eels of Japanese eel (*Anguilla japonica*) in the Changjiang (Yangtze) River estuary, and to investigate the nutritional requirements of wild glass eel, they were sampled in the adjacent waters of Taizhou and Zhoushan of Zhejiang Province, and Yancheng of Jiangsu Province. Using 16S rDNA amplicon sequencing technology, the dominant bacterial communities and functional differences related to nutrient metabolism in the glass eel intestines were analyzed. Results show that the intestinal bacterial community of glass eels at all the three sites was dominated by Proteobacteria and Actinobacteria, which accounted for 68.7%~82.0% and 4.2%~19.0%, respectively. *Acinetobacter* and *Pseudomonas* were the most abundant genera, accounting for 12.4%~17.3% and 8.07%~13.03%, respectively, being dominant genera constantly. In addition, each site featured rich and unique bacterial groups, including *Aeromonas* in Taizhou, *Endozoicomonas* and *Cutibacterium* in Zhoushan, and *Psychrobacter* and *Empedobacter* in Yancheng, which is believed that different water environment factors resulted in site-specific bacterial groups. *Acinetobacter*, *Pseudomonas*, and *Endozoicomonas* have protein-degrading functions in the intestines of various fish species. *Aeromonas* is a cellulose-degrading bacterium and can also secrete extracellular proteases. Furthermore, we found by functional prediction that the transport and metabolism of amino acids took the highest proportion of the functions, which implies that glass eels could consume more protein-rich components for food during migration. This study unveiled the diversified composition in intestinal microbiota of wild glass eels along offshore in East China, and provided a basis for studying the feeding habits and food composition of wild eels, as well as a reference for the design of bait for the artificial breeding of Japanese eel.

**Keywords:** Japanese eel (*Anguilla japonica*); larvae; intestinal microbiota; microbial diversity; high-throughput sequencing

## Effects of Pond Facility Culture Mode on Growth Performance and Off-flavor Substance Content of Largemouth Bass (*Micropterus salmoides*)

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**Abstract:** This study analyzed the growth performance and the content of off-flavor substances, such as geosmin (GSM) and 2-methylisoborneol (2-MIB) of largemouth bass, and the phytoplankton and physical and chemical indexes of water body under the traditional pond culture mode (TPA), In-pond raceway system (IPRS) and Pond recirculating aquaculture system (PRAS). The results showed that there was no significant difference in the content of 2-MIB in the muscle of largemouth bass at the first sampling in the three modes. At the last sampling, TPA was significantly higher than IPRS and PRAS ( $P<0.05$ ), but there was no significant difference between the latter two models. For GSM, the content of GSM in TPA was significantly higher than IPRS and PRAS at the first sampling ( $P<0.05$ ); similar to 2-MIB, TPA was significantly higher than IPRS and PRAS in late sampling, but there was no significant difference between the two modes. The content of crude fat and crude protein increased significantly with the increase of culture time in TPA and PRAS modes ( $P<0.05$ ). Correlation analysis showed that the content of 2-MIB in the muscle of largemouth bass was significantly positively correlated with total nitrogen, total phosphorus, Cyclotella and Euglenophyta ( $P<0.05$ ), and the content of GSM was significantly positively correlated with Cyclotella, Cyanophyta and Euglenophyta ( $P<0.05$ ). In summary, IPRS and PRAS can effectively reduce the concentration of the off-flavor in fish meat, which is a feasible breeding mode for the aquaculture industry to reduce off-flavor.

**Keywords:** Largemouth bass; breeding mode; off-flavor; algae; nutrients

## Development of a Cardiac Performance-based Measure of Stress Resistance in the Ivory Shell (*Babylonia areolata*)

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**Abstract:** The ivory shells (*Babylonia areolata*), a highly valued shellfish in tropical and subtropical regions, encounter challenges in aquaculture due to seasonal fluctuations in water temperature, typhoons, and heavy rainfall. These natural factors can cause significant variations in water temperature and salinity, thereby affecting reproduction, growth, and even leading to substantial mortality. Reinforcing stress resistance traits through genetic breeding is therefore imperative, while concurrently establishing a rapid and precise assessment methodology for evaluating stress tolerance is pivotal. In this study, non-invasive heart rate detection was employed to investigate alterations in heart performance among ivory shells exposed to high temperatures, low temperatures, and low salinity stress. The Arrhenius breakpoint temperature (ABT) and the zero heart rate flat line temperature (FLT) were utilized to gauge the heat tolerance of ivory shells. We compared the effects of individual size (3 cm, 4 cm, 5 cm), acclimation temperature (26 °C, 28 °C, 30 °C, 32 °C), and sex on ABT and FLT. Results indicated that at an acclimation temperature of 26°C, the basal heart rate was recorded as  $31.69 \pm 0.45$  bpm. Only acclimation temperature significantly influenced ABT ( $P < 0.05$ ), while other factors had minimal effects. At 26°C, the mean values of ABT and FLT were  $35.34 \pm 0.98$  °C and  $39.21 \pm 0.70$  °C, respectively. Under low-temperature stress, the heart rate exhibited three stages with decreasing temperature: weakening, arrhythmia, and recovery to normal. In low-salinity stress, the heart rate demonstrated three stages and two turning points, marked by initial deceleration, acceleration, and subsequent deceleration. Additionally, the cardiac data revealed that the ivory shell employed diverse response mechanisms when faced with varying stress conditions. The findings of this study provide valuable insights into the physiological adaptation mechanisms, thereby holding significant implications for genetic selection and molecular mechanism research pertaining to stress resistance traits.

**Keywords:** *Babylonia areolate*; Cardiac performance; Stress resistance

## The mechanism of shell formation in *Turbo argyrostomus*

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**Abstract:** The warm yellow jade like inner shell in *Turbo argyrostomus* is rare in Gastropoda. However, the gene set responsible for its shell formation remains poorly explored. Here we surveyed microstructure with scanning electron microscope (SEM), hematoxylin-eosin staining (HE) and Alcian blue staining- Periodic Acid-Schiff staining (AB-PAS) used for histological observation, supplemented transcriptomic which provide genetic information for shell formation. The results illustrate that *T. argyrostomus* shell exhibits the prismatic-nacreous-prismatic (P-N-P) microstructure through SEM, and histological observation demonstrated two major cell types, including adipocytes and mucin cells. The transcriptomic analysis revealed 11 biomineralization genes, among which WAP (whey acidic protein), N66, and nacre like are believed to related with the warm yellow jade like and shell microstructure. Domain analysis was conducted on the mucin gene to obtain its function in *T. argyrostomus*, 25.20% biomineralization domain in central mantle (E) vs. foot (F) and 22.39% in edge mantle (E) vs. foot (F), supporting for the relationship between mucins and biomineralization, where mucins are considered as framework protein in shell formation. Moreover, this study has discovered that energy distribution differences between edge mantle and central mantle. This study provided insights for further understanding biomineralization in Gastropoda.

**Keywords:** Transcriptome; Microstructure; *Turbo argyrostomus*; Shell formation

## Transcriptomic Analysis Reveals Distinct Response Strategies to Heat Stress in Two Geographic Populations of the *Babylonia areolata*

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**Abstract:** *Babylonia areolata*, a significant mollusk species under cultivation in China, exhibits two major cultured populations: the Hainan population (HN) and the Thailand population (TG). During the high-temperature cultivation process in summer, these two populations exhibit certain differences in terms of growth rate, survival, and disease resistance. In order to elucidate the molecular regulatory mechanisms underlying the response to high-temperature stress in the Hainan and Thailand populations of the *B. areolata*, we conducted a transcriptomic analysis of gene expression profiles under elevated temperature conditions (32°C). Utilizing Illumina novaseq 6000 sequencing, we obtained a total of 122.47 G raw data and 119.71 G clean data, both exhibiting Q20 and Q30 values exceeding 92%. The analysis of differential expression genes (DEGs) revealed substantial disparities between the two populations. In the HN, a total of 2,264 genes demonstrated differential expression, with 1,366 up-regulated and 898 down-regulated DEGs. Conversely, only 193 DEGs were identified in the TG, including 117 up-regulated and 76 down-regulated DEGs. Notably, the magnitude of DEGs was more pronounced in the HN compared to the TG. The results of KEGG enrichment analysis revealed significant enrichment of DEGs involved in antigen processing and presentation, NF-kappa B signaling pathway, and glycine, serine and threonine metabolism in the HN. Among these DEGs, *aep*, *irak1*, and *traf6* emerged as potential key genes closely associated with immune responses in the HN. However, in the TG, predominantly enriched pathways for DEGs included apoptosis, tumor necrosis factor signaling, protein processing in the endoplasmic reticulum, and antigen processing and presentation. Particularly noteworthy is that several genes closely linked to apoptosis regulation such as *hsp70*, *hsp90*, *traf3*, *pepk*, and *bip* were found within TG. The research findings indicate that the two geographically populations employ different strategies in response to heat stress, with the thermal-tolerant TG adopting more energy-efficient and effective mechanisms to cope with elevated temperatures. These results contribute to the enhancing of molecular mechanisms underlying invertebrate responses to environmental stress, while concurrently providing a theoretical basis for the genetic breeding of thermal tolerance in the *B. areolata*.

**Keyword:** *Babylonia areolata*; Geographically population; Thermal tolerance;

Transcriptome; Molecular mechanisms

## **Seasonal Variation of Microbial Communities in Tilapia Broodstock Ponds and on Surface of Tilapia Eggs**

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**Abstract:** Aquaculture pond is a complex ecosystem where the microorganisms in the sediments, in water and on surface of aquatic animals interact with each other to influence health of aquatic animals. In Hainan, China, Tilapia brood stocks spawn all year around. The eggs were quickly colonized by microorganisms in the pond water/sediments after they were laid. The microbial communities on surface of tilapia eggs would greatly influence the health and hatchability of eggs. To investigate the seasonal variation of microbial communities in tilapia broodstock ponds and on surface of tilapia eggs and their interactions, in this study, 16S rRNA gene high-throughput sequencing was used to explore the characteristics of microbial communities in tilapia broodstock pond sediments/water and on surface of tilapia eggs in spring, summer, autumn, and winter. The results showed that Proteobacteria was the most important phylum in the microbial community of tilapia pond and eggs. Moreover,  $\alpha$  diversity represented the highest bacterial diversity in sediment, followed by water, and the lowest bacterial diversity in fish eggs. The microbial community structure of tilapia eggs is similar to that of pond water and sediment which were observed in the  $\beta$  diversity of microorganisms. The significance difference test between groups revealed that the water, sediment microorganisms and eggs microorganisms in fish pond were relatively stable during seasonal changes. The changes in pond environmental factors were significantly correlated with changes in microbial communities, and temperature, pH, dissolved oxygen, and days-post-transferring may be the main factors affecting bacterial diversity. The results of this research helped to reveal the connection between tilapia eggs and pond microorganisms, providing a basis for producers to regulate pond and fish eggs microorganisms.

**Keywords:** tilapia eggs; aquaculture pond; microbial community; seasonal variation; 16S rRNA

## Analysis of Spatiotemporal Distribution Patterns and Screening of Key Virulence Genes in the Process of *Vibrio Harveyi* Infecting Grouper

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**Abstract:** *Vibrio harveyi* is one of the most serious bacterial pathogens to aquatic animals worldwide. Evidence is mounting that coinfections caused by multiple pathogens are common in nature and can alter the severity of diseases in marine animals. However, bacterial coinfections involving *Vibrio harveyi* have received little attention in mariculture. In this study, the results of pathogen isolation indicated that bacterial coinfection was a common and overlooked risk for hybrid groupers (♀*Epinephelus polyphekadion* × ♂*E. fuscoguttatus*) reared in an industrialized flow-through pattern in Hainan Province. The artificial infection in hybrid groupers revealed that coinfections with *Vibrio harveyi* strain GDH11385 (a serious lethal causative agent to groupers) and other isolated pathogens resulted in higher mortality (46.67%) than infection with strain GDH11385 alone (33.33%), whereas no mortality was observed in single infection with other pathogens. Furthermore, the intestine, liver and spleen of hybrid groupers are target organs for bacterial coinfections involving *Vibrio harveyi*. Based on the infection patterns found in this study, we propose that *Vibrio harveyi* may have a specific spatiotemporal expression pattern of virulence genes when infecting the host, providing a new understanding of the pathogenic mechanism of *Vibrio Harveyi*. Based on the temporal and spatial distribution data of *Vibrio Harveyi* in different organs of *Epinephelus*, the host organ suitable for transcriptome sequencing was identified as the liver. Through interaction transcriptome sequencing, we analyzed the differences in in vivo and in vitro gene expression patterns of *Vibrio Harveyi* in hybrid grouper (♀*Epinephelus fuscoguttatus* × ♂*E. lanceolatu*), and screened potential key virulence (related) genes involved in pathways including type VI secretion system, siderophores biosynthesis and transport, flagella assembly, ribosomes, biofilm formation, and sulfur metabolism.

**Keywords:** *Vibrio Harveyi*; artificial infection; bacterial coinfections; virulence gene; hybrid groupers

## Enhancing Microalgae Biomass through Fluorescent Carbon Quantum Dots

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**Abstract:** Microalgae, including algae and phytoplankton, are a category of small plant-like organisms known for their rich nutritional value, serving as an ideal food source for aquatic organisms. They play a crucial role as feed in aquaculture and aquatic ecosystems. However, limited light utilization, specifically the absorption of light only in the blue and red ranges, results in inefficient photosynthesis and slow growth of microalgae. Enhancing the growth rate of microalgae is therefore essential for widespread utilization as feed. In this study, we selected *Chlorella vulgaris* as a model microalga. We utilized Triangular Brown Flagellate as a carbon source to successfully synthesize fluorescent carbon quantum dots emitting at a wavelength range of 450-550 nm and 620-730 nm through a one-pot method. Specially, the red light emitted is perfectly matched with the absorption spectrum of chloroplasts, which greatly promotes the growth of chlorella. By optimizing the amount of carbon dots used, co-cultivation with microalgae at a concentration of 50 mg/L significantly increased the biomass (by 663.3%), carbohydrate (by 117.7%), and proteins (by 35.4%) of microalgae. This study's development of an inorganic material-microalgae hybrid system provides a novel approach to addressing the low photosynthetic efficiency of microalgae. By introducing fluorescent quantum dots, we successfully expanded the range of light utilization by microalgae, thereby significantly enhancing their biosynthetic capabilities. This innovative approach offers a promising solution for sustainable aquaculture and the maintenance of aquatic ecosystems.

**Keywords:** Carbon quantum dots; *Chlorella*; Biomass

## The Influence of Stocking Density and Feeding Quantity on the Growth Performance and Water Quality Parameters of *Moenkhausia costae*

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**Abstract:** The global ornamental fish industry has exhibited rapid growth, driven by its significant economic value. The tropical freshwater fish *Moenkhausia costae* is a highly sought-after ornamental species with substantial market worth. The present study conducted a 60-day culture experiment in a self-designed water circulation system to investigate the impacts of stocking density (1, 2, 3 cm/L) and feeding quantity (3%, 5%, 7% of the weight) on the growth performance, feeding behavior, and water environment of *M. costae*. Our results suggested that the stocking density and feeding quantity group (3 cm/L and 7%) had a significant impact on the growth indices of *M. costae* ( $P < 0.05$ ). The highest weight gain ratio (WGR,  $517.96 \pm 56.09$  %) and weight-specific growth rate (W\_SGR,  $3.03 \pm 0.15$  %/d) with the lowest feed conversion ratio (FCR,  $1.08 \pm 0.02$  %) were observed at low stocking density and feeding quantity group (1 cm/L and of 3%). The daily feed intake (FI) remained stable at approximately 3.5%. Furthermore, the interaction between stocking density and feeding quantity affected FCR in *M. costae*, while water concentrations of  $\text{NH}_3\text{-N}$  and  $\text{NO}_2\text{-N}$  increased with increasing stocking density and feeding quantity levels. High density and high feeding quantity resulted in higher concentrations of  $\text{NH}_3\text{-N}$  ( $0.56 \pm 0.07$  mg/L),  $\text{NO}_2\text{-N}$  ( $0.033 \pm 0.003$  mg/L), oxygen demand (COD) ( $16.95 \pm 0.38$  mg/L), and total nitrogen (TN) ( $7.70 \pm 0.31$  mg/L). The length-weight relationship was fitted using a power function to derive the equation  $W = 0.0165 \times L^{2.5331}$  ( $n = 100$ ,  $R^2 = 0.9873$ ). In our custom-designed recirculating aquaculture system, the optimum breeding strategy for *M. costae* entailed a low stocking density and feeding quantity (1 cm/L and 3%). This study enhances our comprehension of the biological characteristics of *M. costae* and the carrying capacity of recirculating aquaculture systems, thereby paving the way for the development and refinement of large-scale farming techniques for *M. costae* while also providing a foundation for artificial breeding.

**Keywords:** *Moenkhausia costae*; Stocking density; Feeding quantity; Growth performance; Water quality parameters

## Otolith Microstructure And Growth And Developmental Characteristics Of Early Individuals Of The Bluespot Mullet *Crenimugil seheli*

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**Abstract:** Early life history traits (ELHTs) are key to understand recruitment patterns in marine animals. At present, the use of otolith microstructure characteristics to study the early life history of fish has been quite mature. The Bluespot mullet (*Crenimugil seheli*) is an economic species widely distributed in the Indian and Western Pacific Oceans, but there is a gap in research on otolith microstructure and growth and developmental characteristics of its early life history.

In this study, we investigated the otolith microstructure and early life history of 113 Bluespot mullet (*Crenimugil seheli*) collected from the surf zone of Gaolong Bay, Wenchang City, Hainan Province, China, in 2021-2022. Preliminary results showed that the distribution of standard length of the Bluespot mullet (*Crenimugil seheli*) ranged from 8.87 to 45.74 mm, with a mean standard length of  $26.53 \pm 6.78$  mm, and that the distribution of body weight ranged from 0.0075 to 1.9060 g, with a mean body weight of  $0.4452 \pm 0.3634$  g. A power function was used to fit the regression equation of standard length to body weight in the Bluespot mullet (*Crenimugil seheli*), and the result was  $W = 3.9 \times 10^{-6} L^{3.1731}$  ( $R^2 = 0.9516$ ,  $n = 113$ ). The expected findings of this study are expected to fill the gaps in the study of otolith microstructure and growth and developmental characteristics of the early life history stages of the Bluespot mullet (*Crenimugil seheli*), and to provide a scientific basis for the development and utilization of fishery resources as well as the formulation of conservation policies.

**Keywords:** Otolith microstructure; Early life history; *Crenimugil seheli*

## Early development and growth morphology of *Terapon jarbua* in the South China Sea

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**Abstract:** *Terapon jarbua* is a high-value fish species in the reef fish, with stocks declining continually due to increasing market demands and unsustainable fishing practices. Managing this resource is difficult due to lack of biological information on local stocks, especially the condition of early stages. This paper utilized otolith to evaluate the age, growth rate and peak breeding period of *Terapon jarbua* larvae and juveniles in the Hainan surf zone, to infer the stage suitable for larval wild fishing of aquaculture production, offer advice for the protection early stage of *Terapon jarbua*.

**Keywords:** *Terapon jarbua*; Early development; Larvae

## Effects of Stocking Density on Growth, Survival and Water Quality of the Mini Parrot Fish (*Archocentrus nigrofasciatus* var. × *Mikrogeophagus ramirezi*)

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**Abstract:** Due to its distinctive appearance, the mini parrot fish (*Archocentrus nigrofasciatus* var. × *Mikrogeophagus ramirezi*) has gained widespread popularity among fish farmers and enthusiasts of ornamental fish worldwide. Although the density of aquatic organisms plays a crucial role in the breeding process, there is limited research available on mini parrots in this regard. Therefore, this study aimed to investigate the impact of stocking density on growth, survival, and water quality of blue parrot fish through a 49-day experiment by implementing four different stocking densities (A: 1.16kg/m<sup>3</sup>, B: 2.32kg/m<sup>3</sup>, C: 3.48kg/m<sup>3</sup>, D: 4.62kg/m<sup>3</sup>). The results indicated that there were no statistically significant differences in wet weight, total length, and body length among the A, B and C groups ( $P > 0.05$ ). However, group D exhibited significantly lower values for wet weight ( $1.91 \pm 0.72$  g), total length ( $3.31 \pm 0.61$  cm), and body length ( $2.46 \pm 0.38$  cm) compared to the A, B and C groups ( $P < 0.05$ ). No significant differences were observed in the length of dorsal fin, anal fin and caudal fin among four groups ( $P > 0.05$ ). Furthermore, the mortality of group D ( $25.8\% \pm 2.5\%$ ) was significantly higher than that of A, B and C groups ( $P < 0.05$ ). Notably, female fish displayed a significantly higher mean total mortality rate ( $11.7 \pm 5.6\%$ ) compared to male fish ( $4.5 \pm 1.0\%$ ) ( $P < 0.05$ ), with the highest recorded mortality rate being 25%. Additionally, there were no significant differences in pH, ammonia nitrogen and nitrite contents among the four groups ( $P > 0.05$ ). The findings demonstrate that stocking density exerted a significant influence on the growth and survival of the mini parrot fish, while adjusting the stocking density can enhance the yield of this species. This study contributes to the theoretical research on ornamental fish and offers valuable insights for large-scale breeding of the mini parrot fish.

**Keywords:** Mini parrot fish; Stocking density; Growth indicators; Water quality indicators

## The Impact of Stocking Densities on the Growth, Feeding and Water Quality *Hyphessobrycon rosaceu*

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**Abstract:** The density plays a crucial role in aquaculture, and maintaining an appropriate stocking density is essential for disease control, reducing the risk of sudden death, and enhancing growth rates. In this study, a 30-day culture experiment was conducted at different densities (D30: 1.02 kg/m<sup>3</sup>, D45: 1.53 kg/m<sup>3</sup>, D60: 2.05 kg/m<sup>3</sup>, D75: 2.56 kg/m<sup>3</sup>, D90: 3.07 kg/m<sup>3</sup>) to investigate the impact of stocking density on the growth, feeding and water quality in cultivation systems of *Hyphessobrycon rosaceus*. The results demonstrated high survival rates throughout the entire experiment for all groups, except for D90 (97.8%), with the remaining groups achieving 100%. Notably, the D45 group exhibited superior performance in terms of body length (BL), body mass (BM), weight gain rate (WGR, 36.88±9.69%), and specific growth rate (SGR, 1.44±0.46%). Conversely, the D90 group displayed the slowest growth rate (SGR=0.62±0.16), significantly lower than that of other groups ( $P < 0.05$ ). Moreover, the feed conversion ratio (FCR) of the D90 group (6.56±2.24) was notably higher compared to both the D45 and D60 groups ( $P < 0.05$ ), while no significant difference in FCR was observed between the minimum FCR of the D60 group (1.87±0.40) and that of the D45 group ( $P > 0.05$ ). Additionally, the concentration of NH<sub>3</sub>-N in D45 was significantly lower than that in D60, D75, and D90, with a value of only 1.39±0.5 mg/L ( $P < 0.05$ ). Moreover, the highest NO<sub>2</sub><sup>-</sup>-N level (0.091±0.023mg/L) was observed in the D90 group, exhibiting significant differences compared to the levels found in the D30 group (0.018±0.005mg/L), D45 group (0.0235±0.0025mg/L), and D60 group (0.017±0.015mg/L) ( $P < 0.05$ ). With the increase in stocking density, the highest NO<sub>3</sub><sup>-</sup>-N concentration was 33.95±4.61 mg/L in the D90 group, which was significantly higher than that in all other groups ( $P < 0.05$ ). However, there was no significant difference between the D45 group with the lowest NO<sub>3</sub><sup>-</sup>-N concentration (13.66±4.15mg/L) and the D30 group with a similar low NO<sub>3</sub><sup>-</sup>-N concentration (14.74±0.55mg/L). It was observed that the optimal stocking density for *H. rosaceus* was found to be at D45, and its growth curve can be described by  $BM=0.0385 \times BL^{2.3401}$  ( $R^2=0.782$ ). This study contributes valuable data to ornamental fish culture and provides a theoretical foundation for large-scale artificial cultivation of *H. rosaceus*.

**Keywords:** *Hyphessobrycon rosaceus*; Stocking density; growth; Feed conversion

ratio; Water quality

## Specific Antagonism of *Pseudoalteromonas piscicida* WCPW15003 Towards *Vibrio harveyi*: Mechanistic Analysis of Antagonistic Actions

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**Abstract:** *Vibrio harveyi* is the dominant pathogen in mariculture, and biocontrol of this pathogen using antagonistic probiotics is a long-standing biological challenge. Here, *Pseudoalteromonas piscicida* WCPW15003 as a probiotic effectively antagonized dominant pathogenic *V. harveyi* in a mariculture, with a growth-of-inhibition ratio of 6.3 h<sup>-1</sup>. The antagonistic activities of cells and intracellular components of WCPW15003 made a greater contribution to the antagonistic process than did extracellular metabolites and caused the dominance of WCPW15003 during the antagonistic process in vitro. WCPW15003 was safe for the pearl gentian grouper ( ♀ *Epinephelus fuscoguttatus* × ♂ *Epinephelus lanceolatus*) and, as a consequence of the antagonistic effect on *V. harveyi*, protected the fish from an immune response *in vivo*. A comprehensive combined proteomics and metabolomics analysis of antagonistic WCPW15003 and pathogenic *V. harveyi* in a coculture compared to a monoculture was performed to investigate the antagonistic molecular mechanisms. The results showed that during the antagonistic process, WCPW15003 in a coculture had significantly downregulated metabolic pathways for histidine metabolism, arginine biosynthesis, and phenylalanine metabolism, and upregulated glycerophospholipid metabolism, leading to a competitive advantage against the co-occurring species, *V. harveyi*. This defined a mechanism by which multi-costimulatory pathways drove *P. piscicida* WCPW15003 against *V. harveyi*.

**Keywords:** antagonistic bacteria; costimulatory pathway; pathogenic *Vibrio harveyi*; proteomic combined metabolomic analysis; recirculating-mariculture system.

## High-throughput Screening of Heat Stress-Related Genes in the Ivory Snail (*Babylonia areolata*) Based on the Yeast Strain INVSc1

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**Abstract:** The ivory snail (*Babylonia areolata*) has emerged as a significant constituent of China's mariculture industry in recent years. In the cultivation process, high temperatures during the summer season play a crucial role in influencing the survival and growth of this species. However, there is limited research on the molecular regulatory mechanisms employed by the ivory snail in response to heat stress. Yeast, a eukaryotic organism, exhibits a gene expression system that bears closer resemblance to that of animals and plants, significantly reducing the likelihood of false positives in genetic screening. In this experiment, individuals from populations in Thailand, Hainan, and their hybrids were collected to construct cDNA libraries following heat stress in the ivory snail. And we utilized a yeast-based high-throughput screening method to investigate the relevant regulatory pathways and key genes involved in response to heat stress. Ultimately, a cDNA library with a total clone number of  $2.208 \times 10^8$  and a reservoir capacity of  $1.104 \times 10^8$  cfu was obtained. After transformation into the *Saccharomyces cerevisiae* INVSc1, the library titer was determined to be  $1.52 \times 10^8$  cfu/ml. Under heat stress at 39°C, a total of 96 positive clones were randomly selected for colony PCR and NGS sequencing, resulting in the identification of 51 genes through sequences alignment. Further analysis revealed that these genes, such as hspa5, dnajb9, hsp90aa1, hspa14, had been reported to be related to high temperature stress, and were mainly concentrated in the KEGG pathways of protein processing in endoplasmic reticulum, ribosome, ubiquitin mediated proteolysis. The pivotal role of 51 genes in response to heat stress was further substantiated through the implementation of a retransformation experiment. The findings of this study contribute to a deeper comprehension of the molecular regulatory mechanism employed by mollusks in adapting to adverse environments, thereby playing a pivotal role in facilitating genetic selection for thermal tolerance in ivory snails.

**Keywords:** *Babylonia areolata*; Heat Stress; Engineered Yeast INVSc1; Thermal tolerance

# Exogenous Hormones Induce Liver Injury during Sexual Maturation of Japanese Eel *Anguilla japonica*: A Physiological and Transcriptomic Analysis

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**Abstract:** The liver of Japanese eel *Anguilla japonica* can synthesize vitellogenin and promote energy metabolism during sexual maturation. However, exogenous hormones used to induce sexual maturation in female eels may lead to abnormal liver conditions, such as green liver. The cause, mechanism, and effect on gonad development are still unknown. In this study, female eels injected with exogenous hormones were examined to explore the possible causes and mechanisms of liver injury through biochemical assessments, liver histopathology, ultrastructural analysis and RNA-Seq analysis. The results showed that when female eels reached sexual maturity induced by exogenous hormones, their livers exhibited abnormal coloration and damage. For example, the occurrence of green liver was 53.33%, while the activities of aspartate aminotransferase and alanine aminotransferase were significantly increased, and the contents of total protein and glutathione significantly decreased. Additionally, the activities of superoxide dismutase and glutathione peroxidase were significantly decreased. Meanwhile, the analysis of liver structure also showed that female eels in the hormone group had the phenomenon of lipid deposition and liver injury. Transcriptome analysis showed that steroid biosynthesis and fatty acid synthesis were significantly up-regulated, while key genes involved in lipid metabolism such as ABCA1, ABCG5/8 and LPL were significantly down-regulated. Furthermore, key genes involved in bile acid synthesis, such as FXR, SHP, CYP7A1 and CYP8B1 were inhibited. This indicates that female fish may experience lipid deposition and obstruction of bile acid synthesis.

**Keywords:** Green liver; Lipid deposition; Bile acids; RNA-Seq

**Effects of three nitrogen sources and Sodium bicarbonate on the culture of  
*Platymonas gelgolandica***

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**Abstract:** *Platymonas gelgolandica* is a high-quality natural food. The effects of three nitrogen sources on the growth and reproduction of *Platymonas gelgolandica* under natural light and normal temperature were studied. Water was supplemented with 8.77 g Monopotassium phosphate, in which Phosphorus 2 ppm, Ferric citrate 0.876 g, iron 0.2 ppm and EDTA 2g were used as nutrient mother liquor. Potassium nitrate, ammonium sulfate and urea were used as nitrogen sources, and eight concentration gradient media were prepared with three nitrogen sources, which were respectively 10,20,30,40,50,60,70 and 80 ppm, for 24 groups. The number of algae cells was detected by microscope and blood cell count plate mirror every 24 ho It is known that Sodium bicarbonate has an effect on the growth of algae. The best growth concentration of *Platymonas gelgolandica* was used as the medium, and the same suitable concentration of Sodium bicarbonate solution was added to the experiment, to study the effects of Sodium bicarbonate on the growth and reproduction of *Platymonas gelgolandica*. It was found that Sodium bicarbonate could promote the growth of *Platymonas gelgolandica*. It provided carbon source for the algae cells to promote photosynthesis, and could increase the number of the final algae cells. At the same time, we found that Sodium bicarbonate had different promoting effects when mixed with different nitrogen sources in the same environment, and urea had better effect than other nitrogen sources in the medium without Sodium bicarbonate; Sodium bicarbonate reacts with ammonium sulfate to reduce the effect of fertilization, and potassium nitrate works best in the experiment.

**Keywords:** *Platymonas gelgolandica*; Ammonium sulfate; Potassium nitrate; Sodium bicarbonate; Cell number

## **Session 3 Nutrition and Feed**

### **专题三：营养与饲料**

## Co-culture of Hybrid Catfish (*Clarias macrocephalus* × *Clarias gariepinus*) and Carps

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**Abstract:** An experiment was conducted for 112 days to evaluate the growth performance of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) and carps (Silver carp *Hypophthalmus molitrix*, Rohu *Labeo rohita* and Mrigal *Cirrhinus mrigala*) in three different stocking ratios of 3.2:1, 4.8:1 and 6.4:1, and their effects on water quality and nutrient budget. Hybrid catfish were stocked in a 1.2 m<sup>3</sup> cage at a density of 100, 150 and 200 fish/cage. Fish were fed on pellet feed at a rate of 2% body weight. Carps were stocked at a rate of 5000 fish/ha in 250 m<sup>2</sup> ponds. The ratio of carps was 1:1:1. Carps were grown solely on natural food produced by nutrient wastes from caged catfish. Ponds were fertilized prior to stocking at a rate of 4 kg N/ha/day and 1 kg P/ha/day using urea and TSP. Net fish yield of hybrid catfish (28.7 kg/cage), net fish yield of carps (13.3 kg/pond) and combined net yield of caged catfish and pond carps (127.9 kg/pond) were significantly higher ( $P < 0.05$ ) in higher stocking density of catfish. Stocking ratio of 6.4:1 appeared to be the best stocking ratio. Stocking ratio did not affect water quality ( $P > 0.05$ ). Hybrid catfish culture released 61-64% N and 76-79% P, out of which carps recovered 3.1-3.8% N and 2.4-2.9% P, and 1.5-2.3% N and 1.3-14.0% P were lost to the water column while rest nutrients were unaccounted for. Nutrients derived from the water was equal to fertilization rate of 0.9-1.6 kg N/ha/day and 0.3-0.5 kg P/ha/day which were grossly inadequate for phytoplankton production and to support the growth of herbivore carps.

**Keywords:** Hybrid catfish; Carp; Net fish yield; Nutrient budget

## Partial Replacement of Trash Fish with Formulated Novel Feed for Soft-shell Mud Crab Cage Farming in Daily and Alternate-day Feeding Regime in Bangladesh

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**Abstract:** The mud crab (*Scylla olivacea*) is one of the most promising and fast-growing species after the tiger shrimps in Bangladesh due to its high demand in foreign markets. Along with shrimp and prawn farming, crab culture is becoming popular in Bangladesh due to its hardy nature, easy farming techniques, low investment and lower susceptibility to diseases. Soft-shell mud-crab farming process involving collecting crabs immediately after they molt so they have an unconsolidated exoskeleton. This farming practices initiated in the south-east coastal floodplain and then spread in the main shrimp farming area the south-west coastal area of Bangladesh. Mud crabs are fed with low-quality, unprocessed feeds such as trash fish, mollusks, which may deteriorate water quality. These trash fish are hard to find and sometimes compete with the food fish that poor people eat. Therefore, feeding with formulated feed can be an alternative way to avoid such problems. The purpose of this study was to find out how crabs are used as feed for soft-shell mud crab farming when giving every day or every other day. The 45% protein contained research diet's ingredients was fish meal 50%, de-oiled soyabean meal 19%, full fat soybean meal 5%, rape seed meal 7%, wheat flour 16.8%, di-calcium phosphate 0.5%, vitamin-mineral premix 0.1%, choline chloride 0.1%, fish oil 0.5%, salt 0.5%, and pellet binder 0.5%. The research diet comprised 45 % protein and trash fish (*Tongue sole*) contained 17% protein. The water stability, fragility, sinking rate, of feed tested accordingly. The experiment was conducted in two different phases in the first phase for each treatment 32 plastic cages (15 cm L x 12 cm W x 10 cm H) were used for 'one cage one crab' soft-shell crab farming. In the regular feeding trail 5 treatments comprised T1 (crab fed on 100% trash fish), T2 (crab fed on 75% trash fish +25% novel formulated feed), T3 (crab fed on 50% trash fish +50% novel formulated feed), T4 (crab fed on 25% trash fish +75% novel formulated feed) and T5 (crab fed on 100% novel formulated feed). In the 2<sup>nd</sup> phase the alternate day feeding trial comprised four treatments T1 (crab fed on trash fish daily), T2 (crab fed on trash fish alternative day), T3 (crab fed on formulated day daily) and T4 (crab fed on formulated

feed in alternate day). Each treatments comprised 28 cages with the same sizes of phase-1. Water quality factors, were checked every week and found to be within the acceptable range. In the phase-1 the combination of trash fish and formulated feed shown a superior growth, survival and molting rate. In phase-2 feeding every day with trash fish shown higher yield however alternate day shown a better survival. The mud crab was stocked from the wild however hatchery originated artificial feed dependent soft-shell mud crab farming in the cage may produce much better outcomes. However, utilization of novel formulated feed for mud crab farming will bring a revolutionary change in the coastal landscapes of Bangladesh and wider afield.

**Keywords:** mud crab; soft-shell; novel feed; coastal; Bangladesh

## **Inositol Inclusion Affect Growth, Body Composition, Antioxidant Performance and Lipid Metabolism of Largemouth Bass *Micropterus salmoides***

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**Abstract:** The present study explored effects of inositol on largemouth bass (*Micropterus salmoides*), six iso-nitrogenous and isolipidic diets containing 0 mg/kg (G1, control), 125 mg/kg (G2), 250 mg/kg (G3), 375 mg/kg (G4), 500 mg/kg (G5) and 625 mg/kg (G6) inositol supplementation were prepared and fed to cultured fish (initial average weight: 110 ± 1g) for 8 weeks in recirculating aquaculture systems. The results indicated that compared with G1 group, the WGR, SGR, and FE in the G3 group were significantly higher ( $P < 0.05$ ). The crude lipid content of the whole fish and the liver of cultured fish was significantly reduced with inositol inclusion ( $P < 0.05$ ). However, no effects on moisture, crude protein, and ash contents in fish were observed among different groups ( $P > 0.05$ ). Dietary inositol supplementation larger than 250 mg/kg decreased muscular crude protein and total lipid contents ( $P < 0.05$ ). With the increase of dietary inositol supplemental level, serum TG and TC contents reached the maximum value in the G3 group ( $P < 0.05$ ). Additionally, serum LDL-C in G2, G3, G4, and G5 groups was significantly up-regulated and there was no significant change in serum HDL-C among the treatments. Inositol inclusion also significantly reduced serum AKP, ALT, and AST activities as well as serum MDA content but significantly increased serum CAT, SOD, and T-AOC activities. Compared with the control group, the activities of hepatic TL and LPL of the G3, G4, and G5 groups were significantly higher. Above all, dietary inositol supplementation could improve growth performance, antioxidant capacity, regulate blood lipids, reduce liver fat content, and improve the liver function of largemouth bass.

**Keywords:** Largemouth bass; Inositol; Growth performance; Antioxidant capacity; Lipid metabolism

## **Growth Performance of Nuna Tengra (*Mystus gulio*) at Different Stocking Density in Cage Culture System in Coastal Water of Bangladesh**

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**Abstract:** Cage farming of climate resilient and euryhaline fish species can be the best option to adapt with climate change. Cage aquaculture can play an important role in the overall process of supplying large quantity of fish for all through sustainable production, because of the opportunities for the integration of species and production systems in near shore areas as well as the possibilities for expansion with setting of cages far from the coast. Long whisker catfish (*Mystus gulio*) locally known as “Nuna tengra” is an indigenous euryhaline catfish, primarily grow in coastal waters that lives and enters into the riverine habitat. Nuna tengra, *M. gulio* fetches a high consumer preference in the market in many Asian countries including Bangladesh due to nutritional quality and delicious taste. Fingerling ( $5.5 \pm 0.8$  cm) of Nuna tengra was stocked at the rate of 80/ m<sup>2</sup> (T<sub>1</sub>), 100/m<sup>2</sup> (T<sub>2</sub>), and 120/m<sup>2</sup> (T<sub>3</sub>) fish in total nine cages with three replications respectively. 32% protein contained floating feed was fed and fish was reared for three months in cages. Significant growth variation of Nuna tengra was observed in different stocking density in cages of coastal waters of Bangladesh. The results of the present study will play a key role in ensuring food and nutritional security and income of coastal people and minimize vulnerability of coastal fishing communities in Bangladesh and wider afield.

**Keywords:** Cage farming; Euryhaline cat fish; Sustainable production; Floating feed

# Improvements of GIFT Tilapia Growth, Lipid Metabolism, Digestive Enzyme Activities, and Intestinal Health Through The Response Surface Methodology (RSM) Optimization of *Schizochytrium limacinum* and *Lactococcus lactis* HNL12 Supplementation in Diet

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**Abstract:** To enhance the growth of the tilapia (GIFT), the desirability ( $d$ ) was determined using growth indices, calculated as the product of weight gain rate (WGR)<sup>0.23</sup> × diet coefficient (DC)<sup>0.23</sup> × specific growth rate (SGR)<sup>0.23</sup> × relative growth rate (RGR)<sup>0.23</sup> × condition factor (CF)<sup>0.08</sup> on day 28. Taking the overall desirability ( $D$ ) obtained through  $d$  as the response, the single-factor method and a central composite design (CCD) combined with the response surface method (RSM) were used to optimize the supplementary prescription of *Schizochytrium limacinum* and *Lactococcus lactis*. Subsequently, the tilapia was divided into four groups: the RSM group (1.12% *S. limacinum* combined with  $4.03 \times 10^7$  CFU/g *L. lactis* via the RSM); the S group (1.5% *S. limacinum* via the single-factor experiments); and the L group ( $1 \times 10^7$  CFU/g *L. lactis* via the single-factor experiments); and the control group with PBS. The experiments continued for 28 days, and measurements were taken for the growth, lipid metabolism parameters, digestive enzyme activities, and intestinal morphology parameters of the tilapia. On the 7th day, compared to the control group, the pepsin and  $\alpha$ -amylase ( $\alpha$ -AMS) activities were significantly higher in the RSM and S groups. On the 14th day, compared to the control group, the lipase (LPS), pepsin, trypsin, and  $\alpha$ -AMS activities were significantly higher in the RSM group. On the 28th day, compared to the other three groups, the  $D$ , WGR, DC, SGR, RGR, muscle thickness (MT), and ratio of villus height to crypt depth (VL/CD) were significantly higher in the RSM group. Additionally, the RSM group had significantly higher concentrations of HDL-C compared to the control group. The findings demonstrate that the supplementary prescription of 1.12% *S. limacinum* combined with  $4.03 \times 10^7$  CFU/g *L. lactis* was superior to *S. limacinum* or *L. lactis* alone in enhancing the lipid metabolism, digestive enzyme activities, improving intestinal health, and promoting growth in GIFT tilapia.

**Keywords:** GIFT tilapia; RSM; *Schizochytrium limacinum*; *Lactococcus lactis*; supplementary prescription

## **Pathways for High-Quality Development of the Feed Industry Under the Background of Feed Saving**

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**Abstract:** As global food demand continues rising amidst resource and environmental constraints, the importance of the feed industry in livestock operations is becoming more pronounced. This paper aims to explore pathways for the feed industry to achieve high-quality development under the context of feed saving. It discusses key strategies for the feed industry to realize quality growth under current limitations of resources and environmental challenges. Firstly, by integrating industry resources, production costs can be reduced and competitiveness and resource utilization efficiency can be improved. Secondly, by developing alternative feed resources, new types of feed supplies are proposed to address shortage issues and ensure stable and diverse provision. In feed formula design and production, technological innovations help enhance core competitiveness. With support from research institutions and governments, the feed industry accelerates innovation by promoting research and development and application of novel feed materials. Finally, taking aquaculture as an example, the paper explores how alternative feeds can promote feed-saving and eco-friendly development through efficient resource utilization. In conclusion, this paper provides multiple research directions and strategic proposals for high-quality growth of the feed sector, offering valuable references for the industry's sustainable development.

**Keywords:** Feed Industry; Feed Saving; Development Path

## Effects of *Bacillus licheniformis* on Growth Performance, Meat Quality and Intestinal Microflora of Grass Carp (*Ctenopharyngodon idella*)

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**Abstract:** *Streptomyces* spp. is a major producers of off-flavor in aquaculture, and *Bacillus licheniformis* BL23 was screened to inhibit the growth of *Streptomyces*. This study aimed to explore the effects of BL23 on the growth performance, meat quality and intestinal microflora of grass carp exposed to a culture system with off-flavor-producing *Streptomyces*. The experimental grass carp (25.00±0.01g) was divided into four groups, and the addition of BL23 and *Streptomyces* was as follows: A (*Streptomyces*), B (*Streptomyces*+ BL23), C (blank) and D (BL23). The concentration of *Streptomyces* in water was 10<sup>6</sup>CFU/ml, and the concentration of BL23 in feed was 10<sup>7</sup>CFU/g, during which commercial feed was fed. The results showed that the final body weight, weight gain rate, specific growth rate and fatness of the group added with BL23 were significantly higher than those of other groups ( $P<0.05$ ). The BL23 also improved the meat texture, and the springiness, hardness and chewiness of fish significantly in group D than others ( $P<0.05$ ). No significant difference of moisture, crude lipid, crude protein and ash content was observed. Applying BL23 together with fish feed could prevent the off-flavor in fish. The content of geosmin, a major off-flavor compound, in group D was significantly lower than that in other groups ( $P<0.05$ ), and the content of 2-methylisobcmol, the other major off-flavor compound, in group B and D was lower than that in other groups. The intestinal microflora result showed that the proportion of *Proteobacteria* and *Actinomycetes* in the intestinal tract of grass carp in groups B and D decreased significantly ( $P<0.05$ ), while the proportion of *Firmicutes* and *Bacteroides* increased significantly. It is implied that adding 10<sup>7</sup>CFU/g *Bacillus licheniformis* into the feed could improve the growth performance and meat quality, prevent off-flavor contamination, and optimize the intestinal microbiota of grass carp.

**Keywords:** grass carp ; *Bacillus licheniformis* ; growth ; meat quality ; intestinal microbiota

**Dietary Sodium Diacetate Inclusion Relieved Hepatic Glycogen Deposition,  
Oxidative Stress, and Intestinal Microbial Imbalance of Largemouth Bass  
(*Micropterus salmoides*) Fed High Dietary Carbohydrate**

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**Abstract:** The present investigation was designed to identify whether sodium diacetate could improve the growth performance, antioxidant capacity, glucose metabolism and intestinal microbiome balance of largemouth bass (*Micropterus salmoides*) feeding high dietary carbohydrate. Three isonitrogen and isolipid diets comprising 0g/kg, 1g/kg, and 2g/kg of sodium diacetate were manufactured. Each diet was fed to 105 juvenile largemouth bass of 5.8g for nine weeks. The results demonstrated that dietary sodium diacetate significantly reduced the content of serum glucose and hepatic glycogen ( $P < 0.05$ ). Meanwhile, dietary sodium diacetate incorporation significantly enhanced the expression of insulin receptor a (*ira*), insulin receptor b (*irb*), phosphoinositide-3-kinase regulatory subunit 1 (*pi3kr1*) and AKT serine/threonine kinase 1 (*akt1*) ( $P < 0.05$ ), which were associated to the insulin pathway and partly correlated with the increased expression of genes related to glycolysis, such as glycerol kinase (*gk*) and phosphofructokinase liver type (*pfkl*). Moreover, the amount of malondialdehyde (MDA) was dramatically reduced with the addition of dietary sodium diacetate ( $P < 0.05$ ). Additionally, the antioxidant capability was enhanced with the raise of nuclear factor erythroid derived 2 (*nfr2*) and the decrease of kelch like ECH associated protein 1 (*keap1*) being identified as a possible mediator ( $P < 0.05$ ). The analysis of bacterial 16S rRNA V3-4 region indicated that the intestinal microbiota profile was significantly altered at both phylum and genus level with dietary sodium diacetate inclusion ( $P < 0.05$ ), with decreased relative abundance of Mycoplasma, which belonged to Firmicutes. In conclusion, the addition of sodium diacetate to the ration improved glucose metabolism, antioxidant capacity and intestinal flora of largemouth bass and the optimal level of addition was 2 g/kg.

**Keywords:** Sodium diacetate; Glucose metabolism; Antioxidant capacity; Intestinal microbiota; Largemouth bass

## Isolation, Screening and Identification of Intestinal Lipid-lowering Lactic acid Bacteria in Two Species of Mariculture Fish

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**Abstract:** Fatty liver disease is a common nutritional disease in cultured fish in China, which has a great negative impact on aquaculture. In order to screen potential lactic acid bacteria with lipid-lowering effects, 52 strains of lactic acid bacteria were isolated from the intestines of healthy *Siganus fuscescens* and *Trachinotus ovatus* using MRS agar medium. After measuring the hemolysis of these strains, 35 strains of non-hemolytic lactic acid bacteria were obtained. Two potential probiotics with lipid-lowering ability were screened after lipid-lowering activity rescreening (determination of cholesterol-lowering ability *in vitro* and glycerol-lowering ability *in vitro*). Then the tolerance to artificial simulated gastric juice/pancreatic juice, the adhesion ability (surface hydrophobicity and self-aggregation), exoenzyme activity and antagonistic activity against pathogen bacteria were determined for these two strains. Finally, two potential probiotics (LZ8 and F2) with strong lipid-lowering ability *in vitro* were obtained. Strain LZ8 was isolated from the intestine of *Siganus fuscescens* and F2 was isolated from the intestine of *Trachinotus ovatus*. LZ8 and F2 were identified as *Lactococcus lactis* and *Enterococcus faecalis*, respectively, by 16S rRNA sequencing.

**Keywords:** strain screening; lipid-lowering; lactic acid bacteria

# Long-term Phellodendri Cortex Supplementation in Tiger Grouper (*Epinephelus fuscoguttatus*): Dual Effects on Intestinal Health Revealed by Transcriptome Analysis

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**Abstract:** Previous study has shown that short-term (2-week) supplementation of 1% Phellodendri Cortex (PC) significantly promoted the health status of tiger grouper (*Epinephelus fuscoguttatus*). However, its long-term (8-week) effect was still unknown. This study aims to investigate the long-term effects of 1% PC supplementation on tiger grouper, focusing on growth, immunity, disease resistance, and intestinal gene expression. The tiger groupers (initial mean weight 27.5±0.5g) were fed with Phellodendri Cortex supplementation diets and control diets for 8 weeks, respectively. Results indicated that long-term PC supplementation did not affect growth or vibrio disease resistance in tiger grouper. However, transcriptome analysis revealed potential damage to the structural and functional integrity of the grouper's intestine. Conversely, anti-inflammatory and cathepsin inhibition effects were also observed, offering potential benefits in fish enteritis prevention and therapy. Long-term PC supplementation in grouper culture should be applied with caution.

**Keywords:** transcriptome; Phellodendri Cortex; *Epinephelus fuscoguttatus*; long-term supplementation

## **Mannan Oligosaccharide Improves Antioxidant Capacity, Non-specific Immunity And Protection Against *Vibrio* Disease And Typhoon Stress In *Trachinotus Ovatus* Juveniles**

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**Abstract:** This study examined the impact of varying doses of mannan oligosaccharide (MOS) supplementation on the antioxidant defense, non-specific immunity, resistance to typhoon stress, and resistance to disease in *Trachinotus ovatus*. Feeds supplemented with 0%, 0.3%, 0.6%, and 1% MOS were prepared and fed to experimental fish groups C, M1, M2, and M3, respectively. At 28<sup>th</sup> and 56<sup>th</sup> day of the feeding trial, samples from all groups were taken to measure the antioxidant and non-specific immunity parameters of *T. ovatus*. At the same time points (28<sup>th</sup> and 56<sup>th</sup> day), *Vibrio harveyi* challenge tests were also conducted. At 32<sup>th</sup> day, a typhoon hit the experimental site and the survival rates of all groups were calculated. Results showed that after 28 day of MOS supplementation, group M2 and M3 showed considerably higher levels of antioxidant abilities (T-AOC), group M1 and M2 showed significantly enhanced non-specific immunity (AKP, LZM, and TP) levels than those of group C ( $P < 0.05$ ). All MOS groups had significantly lower cumulative mortality following the challenge test and typhoon stress (at day 32) than control group ( $P < 0.05$ ). After 56 days of MOS supplementation, group M2 and M3 showed significantly enhanced antioxidant ability (CAT) comparing to control group. Only one of the non-specific immunity parameters were significantly increased in group M2 and M3 ( $P < 0.05$ ). After challenge test, only group M3 exhibited significantly declined cumulative mortality rate comparing to that of control groups ( $P < 0.05$ ). In summary, supplementation of MOS at dose 0.6% (M2) for 28 days showed the best overall improving effects on the antioxidant capacity, non-specific immunity, disease resistance, and typhoon stress resistance in *T. ovatus* juveniles.

**Keywords:** mannan oligosaccharide; *Trachinotus ovatus*; antioxidant capacity; non-specific immunity; anti-stress

**Effects of Three Strains of Fish-derived *Bacillus Subtilis* on Serum Enzyme Activities, Lipid Metabolism and Disease Resistance of Hybrid Grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂)**

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**Abstract:** Hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) is one of the grouper species of great economic value in the South China Sea. In recent years, probiotics have shown great potential in disease prevention and control in aquaculture. The aim of this study is to investigate the effects of three fish-derived probiotics (*Bacillus subtilis* BS, 6-3-1 and HAINUP40 strains) on serum enzyme activities, lipid metabolism and disease resistance of hybrid grouper. A total of 360 fish were randomly divided into four groups (3 parallel/group, 30 fish/parallel): control group (basal diet); BS group (basal diet +1×10<sup>8</sup>CFU/g BS strain); 6-3-1 group (basal diet +1×10<sup>8</sup>CFU/g 6-3-1 strain); The HAINUP40 group (commercial diet plus 1×10<sup>8</sup>CFU/g HAINUP40 strain) was sampled after a 42-day feeding experiment. The results of the study demonstrated that supplementation with probiotics led to an increase in the proportion of high-density lipoprotein (HDL-C/LDL-C) and enzymatic antioxidant activities (CAT, T-AOC, and GSH-Px) compared to the control group. Additionally, the concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and malondialdehyde (MDA) were significantly reduced ( $P<0.05$ ). In terms of lipid metabolism and apoptosis-related gene expression, the expression of apoptosis-related genes (caspase3, caspase8, caspase9) in the BS group was significantly lower than that in the control group ( $P<0.05$ ). On the other hand, the expression of LPL, ACO-1, G6PD, DGAT2, FAS, ATGL, and other genes related to lipid metabolism in the 6-3-1 group were significantly higher than those in the control group ( $P<0.05$ ). Similarly, the expressions of lipid metabolism-related genes such as PPAR $\alpha$ , ATGL, CPT-1, SERBP-1, AMPK $\alpha$ , FAS, and LPL in the NF4 group were significantly higher than those in the control group ( $P<0.05$ ). Furthermore, both the BS and 6-3-1 strains significantly increased the survival rate of hybrid groupers challenged with *V. haveri* to 72% and 42.9%, respectively. However, there was no significant difference between the HAINUP40 and control groups. In conclusion, the findings of this study indicate that three strains of probiotics have a significant positive impact on liver enzymes, antioxidant activities, lipid metabolism, and resistance to *V. haveri* in hybrid grouper. This study provides a foundation for further research on the mechanism of probiotics in regulating fish hosts and offers a scientific basis for the application of probiotics in the healthy culture of grouper.

**Keywords:** Probiotics; Hybrid grouper; Lipid metabolism; Disease resistance

**Effects of Two Different Fermentation Methods on Nutrient Composition and Bioactive Ingredients of Chinese Herbal Medicine Used in *Babylonia oreolata*(Lamarck) culture**

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**Abstract:** *Babylonia oreolata*(Lamarck) is one of the most economically valuable snail species in the South China Sea. In recent years, Chinese herbal medicines have shown great potential in disease prevention and control of snail culture due to their green and natural characteristics. However, the relatively simple digestive organs of Gastropods and the existence of antinutritional factors and natural structures in Chinese herbal medicine seriously affect the effective use of Chinese herbal medicine in Gastropods. The aim of this study is to investigate the removal effects of anti-nutritional factors and cell wall in Chinese herbal medicine by two kinds of two-step fermentation methods: fermentation method (with mixed probiotics) and fermentation method (with mixed probiotics) with enzymes. After grinding and sterilization, compound Chinese herbal medicine powder, composed of astragalus, bitter melon and pomegranate peel at the ratio of 4:3:3 (w:w:w), was divided into control group, fermented compound Chinese herbal medicine group, and fermented compound Chinese herbal medicine with enzyme group. The fermentation products were dried under vacuum, crushed, and examined for changes in nutrient composition and active ingredients. The results showed that the nutritional composition of compound Chinese herbal medicine changed significantly before and after fermentation. Compared with the control group, the fermented compound Chinese herbal medicine group and the fermented compound Chinese herbal medicine with enzyme group showed significantly increased crude ash content/crude protein content/total flavonoids content/total alkaloid content/total saponins content/crude polysaccharide content( $P<0.05$ ); and significantly decreased crude fat content/crude fiber content/neutral detergent fiber content/acid detergent fiber content ( $P<0.05$ ). In summary, the two fermentation methods can effectively improve the nutritional composition and bioactive components of compound Chinese herbal medicine, and reduce the content of anti-nutritional factors, and the fermentation method with enzymes was better the fermentation method without enzymes. The two fermentation methods provide a new way for the efficient use of Chinese herbs to prevent diseases and improve production performance in the healthy culture of *Babylonia oreolata*(Lamarck).

**Keywords:** Fermented compound Chinese herbal medicine; *Babylonia oreolata* (Lamarck); Nutrient composition; Bioactive ingredients

## The Effect of Feeding Artesunate on Antioxidant and Non-Specific Immune Indexes of Tilapia against LPS Stress

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**Abstract:** Lipopolysaccharides (LPS) induce release of various inflammatory factors in fish. Artesunate (AR), as an extract of traditional Chinese medicine, has been illustrated to possess rapid antimalarial effects, as well as immune regulation, anti-tumor, liver protection, and anti-inflammatory effects. This study explored the effect of AR on antioxidant and non-specific immune of tilapia against LPS Stress. Control group (CON and CON-LPS groups) were fed basal diet with no AR, and AR groups were fed diets containing 0.01, 0.03, 0.06, 0.09, and 0.12mg/kg feed of AR, respectively. After feeding different diets for 4 weeks, CON-LPS group and AR groups were challenged with LPS (twice per week) for 2 weeks, while CON group were challenged with PBS only. Then samples were taken to measure the potential protective effect of AR against LPS stress. Results showed that LPS significantly reduced the activities of alkaline phosphatase (AKP) and superoxide dismutase (SOD) in tilapia, while alanine aminotransferase (ALT) activity, low density lipoprotein (LDL) activity, total cholesterol (T-CHO), and triglyceride (TG) content were significantly increased. When fed with 0.01, 0.03, 0.06, 0.09, and 0.12mg/kg AR, the AKP activity of tilapia was significantly enhanced than that of the CON-LPS group, and the ALT activity was significantly higher than that of the CON group. However, the ALT activity, SOD activity, LDL content, T-CHO content, and TG content were all lower than those of the CON-LPS group; In 0.06 and 0.09mg/kg AR groups, the SOD activity was significantly higher than that of CON-LPS group, in 0.03 and 0.09mg/kg AR groups significantly higher than the CON group, in 0.12mg/kg AR groups significantly lower than the CON group; in 0.09 and 0.12mg/kg AR groups, ACP activity was significantly higher than that of the CON group and CON-LPS group; in 0.03, 0.06, 0.09, and 0.12mg/kg AR groups, HDL was significantly higher than the CON-LPS group, while in 0.03mg/kg AR groups, it was significantly higher than the CON group; in 0.06 and 0.12mg/kg, T-CHO was significantly lower than the CON group; in 0.06, 0.09, and 0.12mg/kg, the TG and LDL contents were significantly lower than the CON group. The results indicate that LPS reduces the antioxidant capacity of tilapia, which may cause damage to its liver and brain, and increase its susceptibility to disease. However, the intervention of AR increased the antioxidant capacity, enhanced fat metabolism ability, and increased immune activity of tilapia, forming an antagonistic effect against LPS.

**Keywords:** lipopolysaccharide; Artesunate; Tilapia; Antioxidant capacity; Immune activity

## Screening of Algae-bacteria Combinations for Growth and Nutrition of Microalgae and Evaluation of Application Potential in Enhanced Food

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**Abstract:** Microalgae has the advantages of high photosynthetic efficiency, small breeding area, rich nutritional value and environmental purification ability. Among them, there is a kind of food microalgae, which can effectively improve the survival rate of breeding animals and ensure the normal transformation and development of seedlings. However, in the natural state, the growth rate of food microalgae is very slow compared with bacteria and other microorganisms, and due to limited production technology, there are defects of incomplete nutrition and high production cost, which restricts the large-scale production of microalgae. However, the studies of microalgal-bacterial interactions have revealed a significant impact of the mutualistic or parasitic relationships on algal growth. The algal growth, for instance, has been shown to be enhanced by growth promoting factors produced by bacteria, such as indole-3-acetic acid. Vitamin B12 produced by bacteria in algal cultures and bacterial siderophores are also known to be involved in promoting faster microalgal growth. Some probiotics are commonly used in aquaculture production to regulate water quality, such as *Photosynthetic bacteria*, *Bacillus subtilis* and *Lactobacillus sp.* , The use of probiotics must have an impact on the propagation of single-celled algae in water. However, the interaction between probiotics and algae has not been thoroughly studied. Therefore, in this study, food microalgae and probiotics were selected as research objects to explore the effects of probiotics on the growth and nutritional value of food microalgae under different combinations of bacteria and algae and different environmental nutritional conditions, providing a theoretical basis for improving the breeding efficiency of food microalgae and realizing large-scale and high-density breeding of food microalgae to achieve higher economic benefits.

**Keywords:** Microalgae; Microalgae-bacteria interactions; Microalgae production; Aquaculture

## Effects of four artificial diets on the growth, intestinal microorganism composition, and fatty acid profile in *Mastacembelus Armatus*

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**Abstract:** *Mastacembelus armatus* is highly favored by consumers due to its delectable meat and high nutritional value. However, large-scale artificial aquaculture of this species has not yet been fully developed. In this study, the effects of four feed groups, namely artificial diet (A), artificial diet + red worm (B), artificial diet + yellow mealworm (C), and artificial diet + barley worm (D), on the growth performance, intestinal flora composition, and fatty acid profile of juvenile *M. armatus* cultured in a circulating water system were compared after a 60-day breeding period. The results revealed that the wet weight of group D was  $10.44 \pm 9.24$ g, while the body length measured  $14.68 \pm 3.72$ cm. Group D exhibited a significantly higher wet weight compared to other groups ( $P < 0.05$ ), with an approximate increase of 30% in comparison to group A, 21% in comparison to group B, and 11% in comparison to group C. The 16S rRNA sequencing results revealed that the number of operational taxonomic units (OTUs) in groups A, B, C, and D were 41, 42, 73, and 30 respectively. Group C exhibited higher diversity and richness in the intestinal microbial community compared to other groups, while group D displayed the lowest richness. *Staphylococcus* was found to be the predominant bacterial group in group A but was rare in other groups. *Clostridium* emerged as the dominant bacterial group in group B whereas *Ortho-omonas* dominated group D; no obvious dominant bacterial group was observed in group C. The content of muscle fatty acids in groups A and C was the highest in C18:1n9c, and group C was significantly higher than group A. The content of muscle fatty acids in groups B and D was the highest in C18:2n6c, and group D was significantly higher than group B. In general, group D exhibited accelerated trends in both growth rate and the highest C18:2n6c content, while group C demonstrated a superior intestinal microbial community compared to other groups. This study establishes a theoretical foundation for large-scale breeding of *M. armatus* and contributes to the advancement of feed research for aquatic animals.

**Keywords:** *M. armatus*; Artificial diet; Growth; Intestinal microorganism; Fatty acid

# **Session 4 Carbon Sink and Fouling Organisms**

## **专题四：碳汇和污损生物**

## Genomic basis of adaptation in a marine fouling sea squirt (*Styela clava*)

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**Abstract:** Tunicates occupy the evolutionary position at the boundary of invertebrates and vertebrates. It exhibits adaptation to globally environmental conditions. Despite hundreds of years of embryogenesis studies, the genetic basis of the invasive habits of ascidians remains largely unknown. The leathery sea squirt, *Styela clava*, is an important invasive species. We used the chromosomal-level genome and transcriptome of *S. clava* to explore its genomic- and molecular-network-based mechanisms of adaptation to environments. Compared with *Ciona intestinalis* type A (*C. robusta*), the size of the *S. clava* genome was expanded by 2-fold, although the gene number was comparable. An increase in transposon number and variation in dominant types were identified as potential expansion mechanisms. In the *S. clava* genome, the number of genes encoding the heat- shock protein 70 family and members of the complement system was expanded significantly, and cold-shock protein genes were transferred horizontally into the *S. clava* genome from bacteria. The expanded gene families potentially play roles in the adaptation of *S. clava* to its environments. The loss of key genes in the galactan synthesis pathway might explain the distinct tunic structure and hardness compared with the ascidian *Ciona* species. We demonstrated further that the integrated thyroid hormone pathway participated in the regulation of larval metamorphosis that provides *S. clava* with two opportunities for adapting to their environment. Thus, our report of the chromosomal-level leathery sea squirt genome provides a comprehensive genomic basis for the understanding of environmental adaptation in tunicates. In addition, we are screening the anti-fouling molecules from the microbiota of coral based on the signaling that we identified in the genome of *Style*.

## The Impact Of Seaweed Addition On The Carbon Sink Of The Coastal Wetland's Sediment Under Bait Input

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**Abstract:** Residual bait is the primary pollution source of marine aquaculture and significantly impacts the carbon sequestration of coastal wetlands. Adding seaweed can improve aquaculture feed's nutritional structure and enhance feed utilization efficiency. Therefore, it is a current research hotspot for new types of aquaculture feed. However, the impact of seaweed addition on the carbon sequestration of coastal wetland deposits is still unclear. In this study, through 96 days of indoor incubation, we simulated the input of residual bait into the depositional environment of coastal wetlands. We studied the influence of general feed and seaweed composite feed residual bait input on the organic carbon content of the sedimentary environment. Combined with the temporal analysis of the microbial community structure in sediment, we explored the impact and mechanism of seaweed addition on the carbon sequestration of coastal wetland deposits under high residual bait input. In the early incubation stage, the soluble organic carbon in sediment and overlying water in both feed groups rapidly increased. In contrast, the sediment's total organic carbon content rapidly decreased. After 96 days of cultivation, the total organic carbon content in the sediment of the general feed group was significantly lower than that of the control group ( $p < 0.05$ ), while there was no significant difference between the seaweed addition group and the control group ( $p > 0.05$ ). This indicates that the input of residual bait reduces the carbon storage capacity of the depositional environment, while seaweed addition can restore the carbon storage capacity of the depositional environment. Analysis of the microbial community structure showed that the input of both feeds resulted in a significant decrease in microbial community abundance and diversity in sediment. After 6 days, the abundance and diversity of microbial communities showed a clear upward trend. After 48 days, the seaweed addition group had higher abundance and diversity than the general feed group. Co-occurrence network analysis showed differences in network modules between the two feed groups, indicating different module-trait relationships. This study suggests that seaweed addition may affect the carbon storage capacity of high-nutrient depositional environments by changing the microbial community and its functions, providing new ideas for the carbon sink of large seaweed and aquaculture.

**Keywords:** seaweed; carbon sink; coast wetlands; sediment

## The Regulatory Mechanisms of Ascidian Larval Metamorphosis: Insights for Developing Novel Antifouling Strategies

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**Abstract:** The biofouling problem caused by adhesive ascidians is one of the most severe factors leading to substantial economic losses in shellfish aquaculture. However, the current biological approaches to prevent ascidian biofouling are not effective due to a lack of understanding of the molecular mechanisms that mediate ascidian larvae to settle on aquaculture facilities and then undergo metamorphosis to facilitate aggregation. In the present study, we focus on the neuroregulatory system of ascidian larvae and attempt to delineate the significant molecular signaling pathway(s) mediating metamorphosis. Parallely, a systematic screening of active substances from marine natural products is being carried out, which aims to search for effective anti-metamorphosis substances targeting neuroregulatory pathways and having the potential to be developed as antifouling agents. **1)** To investigate the neuroregulatory mechanism of ascidian larval metamorphosis, we identified the putative neurohormones by analyzing the transcriptome of ascidian larvae during metamorphosis. Two evolutionarily conserved neurohormones, gonadotropin-releasing hormone (GnRH) and glycoprotein hormone (GPH), were shown to be highly expressed in the metamorphic stages of larvae and to be capable of activating their cognate G protein-coupled receptors to initiate the canonical Gs-cAMP signaling pathway. By treating the larvae with pharmacological agonists, we proved that either synthesized GnRH peptide or GPH receptor agonist could significantly accelerate larval metamorphosis. The functional characterization of these neurohormones highlights the potential of GnRH and GPH systems being pharmacological targets for metamorphosis inhibition. **2)** To develop novel antifouling agents, we started with a screening of bioactive substances derived from coral symbiotic bacteria. A total of 64 strains of symbiotic bacteria were isolated and cultivated to generate the natural products, followed by the test of their inhibitory effects on larval metamorphosis. The natural products from two strains exhibited high potency on metamorphosis inhibition, suggesting that they can be further separated to obtain bioactive components with the potential of anti-neuroregulation. In summary, our present work provides the basis for elucidating the regulatory mechanisms of ascidian larval metamorphosis and offers insights into developing novel antifouling strategies for ascidian biofouling prevention in aquaculture.

**Keywords:** Ascidian; Metamorphosis; Neurohormone; Natural products; Antifouling

## Carbon sequestration effects and roles in high quality development of fisheries of seaweeds

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**Abstract:** Seaweed plays an important role as a primary producer in the ocean, contributing significantly to the marine environmental problems such as ocean acidification, hypoxia, eutrophication, harmful algal blooms, carbon capture and sequestration. The recent results show that cultivated seaweed such as *Gracilaria* and wild *Sargassum* bed can improve DO, pH, decrease N, P concentrations and then purify water quality and improve coastal environment. Based on the seaweed bioremediation technology, resource conservation technology, and ecological enhancement technology, it is possible to increase the resources of both cultivated and wild seaweed, improve marine habitats, conserve fishery resources, and promote the sustainable development of carbon sequestration fisheries. By utilizing seaweed green feed technology, it is able to reduce greenhouse gas (e.g., methane) emissions from livestock animals. Large-scale seaweed cultivation and wild seaweed bed enhancement is an effective approach for developing low-carbon economy, increasing marine C sequestration, solving marine environmental problems and maintaining high quality development of fisheries.

**Keywords:** Seaweed; Carbon sequestration; High quality development of fisheries

## Carbon sink potential and environmental benefits of seaweed: a case study of the seaweed cultivation industry on China coast

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**Abstract:** Initiatives to mitigate climate change have recently aroused great interest in the role of natural carbon sinks in coastal ecosystems. Seaweeds are widely distributed in the world and represent a large biomass. Since they are part of the global carbon reserves, they remove carbon dioxide and influence climate change. Using the recent 20 years of seaweed mariculture in China's coastal areas as a case, this paper quantifies the carbon storage in cultivated seaweed biomass, and evaluates the carbon sink potential, the remediation ability of nitrogen and phosphorus and other environmental benefits. The carbon sink potential of cultivated seaweeds exhibits a rising trend from 2000 to 2019. The total carbon, nitrogen, and phosphorus removal amount of seaweed were  $3,551,754.9 \pm 177,587.75$ ,  $297,772.25 \pm 14,888.61$ ,  $39,967.15 \pm 1,998.36$  tons according to the seaweed cultivation yield in China coast. The total benefit of carbon sequestration was  $22,405.67 \pm 259.59$  million CNY, and the average benefit was  $1,120.28 \pm 263.27$  million CNY, with the average annual growth rate of 4.00%. The total environmental benefit was  $1,962,020.88 \pm 22,747.42$  million CNY, and the average benefit was  $98,103.04 \pm 23,073.08$  million CNY, with the average annual growth rate of 4.00%. The present study illustrates distinctly the ecological value of large-scale cultivated seaweeds in increasing marine carbon sequestration and resolving offshore environmental issues, which is an efficient approach and comparable to other key marine ecosystems. Based on the carbon sink role of seaweed mariculture, and with the goal of global carbon neutrality, more attention should be paid to the carbon sink capacity of large-scale seaweed cultivation industry and be vigorously developed worldwide.

**Keywords:** Seaweed cultivation; Biomass; Carbon sink; Environmental remediation; Environmental benefits

## Investigation and Research on Carbon Sink of Cultured Seaweed

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**Abstract:** Numerous studies have shown that seaweed such as kelp and gracilaria are huge marine carbon sinks. The large-scale cultivation of seaweed can achieve negative carbon dioxide emissions, and has great potential in carbon sequestration (long-term storage of carbon in the ocean) and climate change mitigation. China's seaweed farming scale and production have ranked first in the world, and seaweed farming can become an effective means of reducing emissions and increasing sinks. However, the potential of utilizing seaweed farming to achieve carbon neutrality is still unclear. Most existing researches have insufficient understanding of the carbon sequestration and process of seaweed aquaculture, especially the yield of particulate organic carbon and dissolved organic carbon. Xiapu, Fujian Province, is the largest seaweed farming county (district) in China, with the area and yield of seaweed farming ranking among the top coastal counties and cities in China. In different seasons and different growth stages of seaweed, our laboratory conducted 8 in-site investigations on the carbon sink of cultured kelp and gracilaria, with Xiapu County as the key research area. At the same time, we are also conducting indoor controlled cultivation experiments, photosynthetic carbon fixation experiments, and dissolved organic carbon (DOC) photodegradation experiments in the laboratory. Through these technological means and methods, we analyze and characterize the carbon sink of aquaculture seaweed from different perspectives, including biological carbon storage, environmental carbon storage (including dissolved organic carbon, particulate organic carbon, sedimentary organic carbon, etc.), carbon sequestration rate, organic carbon release rate, inert organic carbon yield, and sea-air flux of CO<sub>2</sub>. Through the above work, we aim to establish scientific and feasible methods and technical regulations for assessing the carbon sink of farming seaweed, and make contributions to quantifying the carbon sink of seaweed aquaculture.

**Keywords:** Seaweed; Carbon sink; Cultivation

## The Molecular Phylogeny of Caenogastropoda (Mollusca, Gastropoda) Based on Mitochondrial Genomes and Nuclear Genes

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**Abstract:** Comprising about 60% of gastropod diversity, caenogastropods display almost all kinds of shell forms and include many commercially important marine groups. Although the monophyly of Caenogastropoda has been widely accepted, the internal phylogenetic relationships of Caenogastropoda remain unclear. In order to further resolve the caenogastropod phylogenies, we retrieved complete mitogenomes and three nuclear fragments (18S, 28S ribosomal RNA and Histone 3 genes) from newly sequenced high-throughput data of 27 caenogastropods belonging to eight superfamilies. All newly sequenced mitogenomes stuck to the consensus gene order of caenogastropods, except for those of Vanikoroidea, Vermetoidea and Cerithioidea that involved not only the rearrangements of transfer RNA genes but also the protein-coding genes. Phylogenetic relationships were reconstructed based on the nucleotide sequences of both mitogenomic and nuclear datasets using probabilistic methods. The reconstructed mitogenomic phylogeny suggested the monophylies of Architaenioglossa, Sorbeoconcha, Hypsogastropoda and the siphonate clade, regardless of Vanikoroidea, Vermetoidea and Epitonioidea with long branches. The phylogenetic positions of the latter three groups were not able to be determined in the mitogenomic tree due to long-branch attraction artifacts, whereas the nuclear dataset recovered Vanikoroidea as sister to Rissooidea + Truncatelloidea and thus supported the validity of Rissoiform clade. The present study also recovered the close affinity of Cypraeoidea, Ficoidea, Tonnoidea, and Neogastropoda, which could be supported by the presence of a pleurembolic proboscis. The monophyly of Neogastropoda was not supported, as Cancellariidae was recovered as sister to the limpet shaped group Calyptraeidea, and (Tonnoidea + Ficoidea) as sister to the remaining neogastropods. The present study could provide important information to better understand the evolution of caenogastropods, as well as to better protect and utilize the diverse and economically significant marine resources.

**Keywords:** Caenogastropoda; Phylogeny; Sorbeoconcha; Hypsogastropoda; Mitochondrial genomes

## Effects of Seaweed on DOC Content in Seawater—A Case Study of *Eucheuma gelatinae* and *Sargassum polycystum*

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**Abstract:** Seaweeds are important primary producers in marine ecosystems, with high productivity and the ability to absorb essential elements from the environment. Seaweeds contribute to the restoration, improvement, and protection of marine ecosystems, playing a key role as one of the major contributors to the marine carbon sink. Seaweeds convert atmospheric CO<sub>2</sub> into carbon stored in their tissues and transform dissolved inorganic carbon (DIC) from the seawater into dissolved organic carbon (DOC) via photosynthesis. The present study investigated the DOC concentration variations in water during different growth cycles of *Eucheuma gelatinae* and *Sargassum polycystum*, in order to understand the impact of seaweed growing on water DOC content and its role in the marine carbon cycle. The samples were collected to measure DOC, nitrogen(N), and phosphorus(P) concentrations of seawater in the growth areas of *E. gelatinae* and *S. polycystum* from April to June 2023 in Wenchang and Changjiang cities, Hainan Province. The results showed that the growth periods of seaweeds released DOC, leading to an increased of DOC concentration in seawater. In the growth area of *S. polycystum*, the DOC concentration increased gradually, reaching a minimum of 15.66 mg/L in May and a maximum of 22.39 mg/L in June. In the cultivation area of *E. gelatinae*, the DOC concentration increased gradually from April, reaching a maximum 56.09 mg/L in June. There were differences in the absorption capacity of N and P between *E. gelatinae* and *S. polycystum*. *E. gelatinae* demonstrated better absorption of N and P, significantly improving the water quality in the cultivation area. Therefore, promoting the large-scale seaweed cultivation is a crucial measure for marine ecosystem restoration and marine carbon sink development.

**Keywords:** Seaweed; Dissolved inorganic carbon; Dissolved organic carbon; Carbon sink

## C and N Budgets of Green Mussels *Perna viridis* at Different Microalgae and Food Concentrations

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**Abstract:** In order to estimate the combined effects of microalgae species and food concentration on the carbon and nitrogen budget of the green mussel *Perna viridis*. Green mussels were exposed to nine combined treatments with three food concentration levels (2.2, 4.4, and 6.6 mg·l<sup>-1</sup>) × three microalgae species (*I. galbana*, *C. meülleri*, and *P. subcordiformis*) for 7 d. We found that the ingestion carbon (IC) and grow carbon (GC) were significantly increased by elevated food concentration in the experiment. However, growth nitrogen decreased with increasing food concentration in the treatment group fed *C. meüller*, which was not observed under the other two microalgae species. The respiration carbon (RC), ammonia excretion carbon (EC), and feces carbon (FC), although significantly affected by food concentration, behaved differently under different food concentrations. Interestingly, the effects of food concentration, microalgae species, and their interaction on calcification carbon were not found to be significant. These findings have important implications for the role of shellfish aquaculture as a carbon sink.

**Keywords:** Carbon budgets; *Perna viridis*; Respiration rate

## A Study on the Price of Carbon Sinks in China's Marine Fisheries

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**Abstract:** The carbon sink function of cultured shellfish and algae is an important aspect of marine ecosystem function. Their significant carbon sequestration potential provides a valuable means to enhance ecological sequestration. Studying the price mechanism for ecological sequestration of shellfish and algae will facilitate the participation of marine fishery carbon sink in domestic voluntary emission reduction market trading and international carbon emission rights trading. This will enable the realization of the ecological value of China's marine fisheries. Currently, there is a dearth of research on the pricing of carbon sinks in marine fisheries. The pricing of carbon sinks in marine fisheries is constrained by the imperfect measurement and accounting methods used for carbon sinks. Based on a review of relevant literature, this study proposes the intrinsic value of China's fishery carbon sink. Specifically, it aims to determine the shadow price of China's marine fishery carbon sink by analyzing the data on shellfish and algae culture in marine fisheries from 1979 to 2022. The study establishes a model that goes beyond logarithmic production and utilizes ridge regression estimation and testing. Finally, the shadow price of China's marine fishery carbon sink is calculated for the period from 1979 to 2022. Furthermore, this study measures the extent of price distortion of carbon sink factors in China's marine fisheries based on the current price of CER in China's carbon trading market. It analyzes the reasons and economic effects of the price distortion of carbon sink elements in China's marine fisheries and proposes relevant policy suggestions to address this distortion.

**Keywords:** Marine aquaculture; carbon sink; ridge regression; price mechanism

# **Session 5 Health and Environment Management**

## **专题五：健康和环境管理**

## Assessment of Biofouling on Lobster Cages In Xuan Dai Bay, Phu Yen Province, Viet Nam

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**Abstract:** This study aimed to investigate the composition and biomass of fouling algae and fauna on lobster cages in Xuan Dai Bay. Monthly samples were collected from the sides and bottom of five lobster cages in Xuan Dai Bay, Phu Yen Province, from July to December 2017. The results revealed that the fouling algae consisted of 20 genera from two families. Bacillariophyceae was the dominant group with 19 genera, accounting for 95% of the total, while one genus belonged to Chrysophyceae. The fouling fauna comprised 21 species belonging to three classes. Among these, 14 species were classified under seven families of bivalve mollusks (Anomiidae, Arcticidae, Limidae, Mytilidae, Ostreidae, Pinnidae, and Veneridae), representing 67% of the total. Additionally, six families of Gastropoda (Cystiscidae, Buccinidae, Neritidae, Thiaridae, Pyramidellidae, and Vermetidae) accounted for 28% of the total fouling fauna. The highest biomass of fouling algae was observed in September, with 25,140 cells per square decimeter (dm<sup>2</sup>), while the lowest biomass was recorded in November, with 4,169 cells/dm<sup>2</sup>. In terms of fouling fauna, the highest density was observed in July, with 81 individuals/dm<sup>2</sup>, whereas the lowest density was recorded in December, with 10 individuals/dm<sup>2</sup>. Canonical correlation analysis (CCA) revealed that the biofouling communities were influenced by various factors, including pH, salinity, NH<sub>3</sub>, NO<sub>2</sub>, total nitrogen, soluble phosphorus, and total phosphorus.

**Keywords:** Algae; Bivalve mollusc; Fouling; Lobster cage

## Overview of Cage Aquaculture Practices, Benefits and Challenges on Africa Waters Bodies

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**Abstract:** Cage aquaculture is highly preferred due to higher production per unit volume of water, lower costs of investment, and simpler routine farm management procedures compared to pond system. In the 1980s cage culture was first used on a trial basis in sub-Saharan Africa. Over the past 20 years, a small number of prosperous fresh water cage culture operations have started to emerge in Egypt, Rwanda, Kenya, Uganda, Tanzania, Ghana Malawi, Zambia and Zimbabwe. Brackish and marine cage culture also offers a lot of potential, although this subsector hasn't seen any significant commercial growth to date. In 2019, 263 cage aquaculture installations on the African inland waters on 18 water bodies, within eight countries with an estimated 20,114 cages were reported. The lakes Victoria, Kariba, Volta, and River Volta, which together account for 82.9% of all cage aquaculture installations regarded as sub-Saharan Africa's principal cage aquaculture regions (Fig 1). Except few small-scale trials with North African catfish (*Clarias gariepinus*), almost all farms in Sub-Saharan Africa and Egypt grow Nile tilapia (*Oreochromis niloticus*). More than 223,150 tonnes of fish are produced yearly from eight African countries through cage aquaculture. The expansion of cage culture in African provides job opportunity for both skilled and unskilled workers, nutritious food and foreign currency. The escaping non-native strains of tilapia in Lake Volta, and the occurrence a risky Tilapia lake virus (*Syncytial hepatitis*) which has potential to wipe out entire populations in both wild and farmed Nile tilapia on Lake Victoria are threats coming with the expansion of cage aquaculture in Africa. In addition, the installations of 138 cage aquacultures were found in contrary with best cage culture practices. To sustain cage aquaculture development and maintain in harmony with other water uses developers must strictly abide by best practices. Hence, exclusion of protected areas and small lakes (average depth 5 m or less) should be done as well as Environmental Impact Assessment should be conducted before establishing the cage farms.

**Keywords:** Africa; Cage Aquaculture; Production; Threats

## Feed and Feeding Management in Aquaculture: The Concept, Technological Scheme and Practices

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**Abstract:** The percentage of fed aquaculture in aquaculture production continuously increased with the fast expansion of global aquaculture industry, and diet becomes the determinant factor influencing benefits of aquaculture practices. Feed and feeding management is defined as the way to improve survival and growth of the farmed animals and reduce production cost and negative impacts on natural resources and environment of aquaculture practices by integrating feed management (using high quality and cost-effective diet formula) and feeding management (using the optimal feeding frequency and ration size), and has been recognized one of the most important aspect in optimization of aquaculture model. Although numerous attempts focused on nutrient requirement, dietary fish meal replacement or feeding management of aquatic animals, however, only few studies concern the integration of feed management and feeding management for the aquatic animals with commercial importance to fed aquaculture under specific aquaculture conditions due to the research of nutrient requirement, dietary fish meal replacement and feeding management belong to nutrition, feed science and feeding ecology area, respectively. In this paper, the author proposed and defined the concept of feed and feeding management, on basis of the research on feed management and feeding management, and elucidated the role of feed and feeding management in optimization of aquaculture model. The author indicates that the diagram for optimizing feed and feeding management include: (1) determining nutrient requirements of the farmed animals, and (2) determining the high quality and cost-effective diet formula with feed ingredients that are rich in nutrients, deficient in antinutritional factors and constant in supply to meet nutrient requirement of the farmed animals, (3) determining the optimal feeding frequency and ration size for the farmed animals fed with the diets manufactured according to the high quality and cost-effective diet formula, and (4) determining the scheme of feed and feeding management by integrating feed management and feeding management, and assessing impact of optimizing the scheme of feed and feeding management on production, production cost, natural resource depletion and environmental pollution. The author retrospect the researches on feed and feeding management for cuneate drum, golden pompano, largemouth bass and large yellow croaker and preliminary results of the schemes of feed and feeding management, including the optimal dietary protein and lipid levels, low fish meal diet formula and the optimal feeding frequency and ration size. Finally, the author display the challenges to optimize the scheme of feed and feeding management, and propose the way to solve the problems, including (1) appreciating the difference in demand of species and genetic background as well as aquaculture environments on the scheme of feed and feeding management, (2) appreciating the reliability and accuracy of the data of the research on nutrient requirement of the farmed animals and feed ingredient assessment to consolidate the database for diet formula design, (3) appreciating the interactive effect between feed management and feeding management on growth of the farmed animals, (4)

appreciating the importance and necessity of long-term field experiment in evaluating the benefits of feed and feeding management on farming practices, particularly the value of aquaculture ecosystem services. The author predicts that feed and feeding management will become an important area in optimization of aquaculture model and informationization of aquaculture management in the future.

**Keywords:** feed formula; feeding management; feed and feeding management; growth; feed cost; waste outputs

## Mechanism Study on the Effect of Nicotinamide and $\beta$ -Arbutin on the Body Color of Leopard Coral Grouper

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**Abstract:** Body color is an important economic trait of the leopard coral grouper (*Plectropomus leopardus*). The purpose of this study is to highlight erythrophore by reducing the level of melanin in the skin. This study attempted to add 0.5% nicotinamide and 0.5%  $\beta$ -Arbutin to the feed formula was continuously fed for 60 days. The results showed that after feeding, the time for the leopard coral grouper to maintain uniform body color significantly prolonged, and the melanin content in the skin significantly decreased, with a decrease in the number of melanin particles. The levels of superoxide dismutase (SOD) and catalase (CAT) in the skin decrease, while the content of malondialdehyde (MDA) increases. The expression of genes Tyr, SOX10, and Mitf related to melanin synthesis, proliferation, and differentiation is reduced. The apoptosis related gene Bax gene is upregulated, while the Bcl-2 gene is downregulated, with Bax/Bcl-2>1. The activity of tyrosinase decreases, while the content of melanin metabolism enzymes increases, and the effect of nicotinamide is better than that of the positive control  $\beta$ -Arbutin. This study provides a new theoretical basis for the color control of the leopard coral grouper.

**Keywords:** Nicotinamide;  $\beta$ -Arbutin; body color; leopard coral grouper; melanin

**Characterization And Expression Analysis of Mitochondrial Localization  
Molecule: NOD-Like Receptor X1 (NlrX1) in mucosal tissues of turbot  
(*Scophthalmus maximus*) following bacterial challenge**

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**Abstract:** The NOD-like receptor X1 (NLRX1) is a member of highly conserved nucleotide-binding domain (NBD)- and leucine-rich-repeat (LRR)-containing family (known as NLR), that localizes to the mitochondrial outer membrane and regulate the innate immunity by interacting with mitochondrial antiviral-signaling protein (MAVS). As one of cytoplasmic PRRs, NLRX1 plays key roles for pathogen recognition, autophagy and regulating of subsequent immune signaling pathways. In this study, we identified the *nlrX1* in turbot as well as its expression profiles in mucosal surfaces following bacterial infection. In our results, the full-length *nlrX1* transcript consists of an open reading frame (ORF) of 4,886 bp encoding the putative peptide of 966 amino acids. The phylogenetic analysis revealed the SmNlrX1 showed the closest relationship to *Cynoglossus semilaevis*. In addition, the NlrX1 mRNA expression could be detected in all the examined tissues, with the most abundant expression level in head kidney, and the lowest expression level in liver. Moreover, NlrX1 showed similar expression patterns following *Vibrio anguillarum* and *Streptococcus iniae* infection, that were both significantly up-regulated following challenge, especially post *S. iniae* challenge. Finally, fluorescence microscopy unveiled that the SmNlrX1 localized to mitochondria in HEK293T by N-terminal mitochondrial targeting sequence. Characterization of NlrX1 might have an important implication in bioenergetic adaptation during metabolic stress, oncogenic transformation and innate immunity and will probably contribute to the development of novel intervention strategies for farming turbot.

**Keywords:** NLRX1; Mucosal tissues; Mitochondrion; *Scophthalmus maximus*; *Vibrio anguillarum*; *Streptococcus iniae*

## Composition and Seasonal Variation of Symbiotic Macrobenthic Fauna in Sponge *Halichondria panicea*

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**Abstract:** The unique canal system of sponges provides refuge for a diverse array of benthic organisms, which exerting a significant influence on the surrounding ecological environment. In this study, we investigated the composition and seasonal variation of symbiotic macrobenthic fauna in the sponge *Halichondria panicea*, aim to get more information of the relationship in sponge and the symbiotic macroorganisms. Monthly analysis and identification of symbiotic macrobenthic biota revealed that the presence of 33 species belonging to 6 classes, 20 families, and 29 genera within the sponge. Notable taxa include crustaceans dominated by *Elasmopus pecteniscrus*; *Corophium kitamorii*; *Ampithoe valida* and *Leptochelia dulia*; polychaetes represented by *Thelepus plagiostoma* and *Haplosyllis spongicola*; as well as a small number of Plesioceda, Polyps, Bivalves and Sea Spiders. The composition of these macrobenthic organisms exhibits remarkable diversity and abundance, with significantly higher habitat density and biomass compared than the surrounding environment. In addition, the benthic organisms inhabiting the sponge body exhibit significant seasonal variations, exemplified by a peak in organism population during November that is nine times higher than the lowest point observed in April. This seasonal difference is related to the difference in species of organisms and sea temperature. Our study shows that sponge can provide shelters for many benthic organisms and additional research is needed to determine the full extent and implications of their ecological effects.

**Keywords:** Sponge; *Halichondria panicea*; symbiotic macrobenthic; seasonal variation

## 17 $\alpha$ -Methyltestosterone Inhibits the PI3K/Akt/FoxO3a Signaling Pathway in the Brain, Leading to Abnormal Gonadal Development in *Gobiocypris rarus*

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**Abstract:** 17 $\alpha$ -Methyltestosterone (MT) is a synthetic androgen that can disrupt the reproductive development of fish, even lead to imbalances in the population structure of fish in the natural environment. *Gobiocypris rarus* is an endemic species in China with a narrow distribution range. The habitat of this fish with high sensitivity to environmental pollutants is easily influenced by external environmental factors. Previous studies have shown that MT can disrupt the reproductive system of *G. rarus* and inhibit the maturation of germ cells. To further investigate how MT regulates gonadal development through HPG axis, *G. rarus* were treated with 0, 25, 50, and 100 ng/L of MT for 7, 14, and 21 days. The data obtained from gonadal histology, reproductive-related genes and hormones in brain, and transcriptome profiling of brain tissue were analyzed. With the increasing exposure time and concentration, worsened gonad degeneration and decreased quantity proportion of mature oocytes were observed. Significant reductions in GnRH, FSH, and LH levels occurred in both male and female *G. rarus* treated with 100 ng/L MT for 14 days, the expression of the *kiss2* gene initially increased and then decreased, while extended treatment up to 21 days resulted in decreased expression of both *kiss1* and *GPR54a*. The expression of *gnrh3*, *gnrhr1*, *gnrhr3*, *fsh $\beta$* , and *cyp19a1b* genes significantly decreased in the brain of male and female *G. rarus* treated with 100 ng/L MT for 14 days. Finally, 2412 and 2509 differentially expressed genes (DEGs) in brain tissue were identified in male and female fish treated with 100 ng/L MT for 14 days respectively. MT suppressed the PI3K/Akt/FoxO3a signaling pathway by upregulating genes such as *foxo3* and *ccnd2*, and downregulating genes such as *pik3c3* and *ccnd1*. The study demonstrates that MT interferes with the levels of GnRH, FSH, and LH in *G. rarus* brains through the PI3K/Akt/FoxO3a signaling pathway, and affects the expression of *gnrh3*, *gnrhr1* and *cyp19a1b* to interfere with the stability of the HPG axis, thus leading to abnormal gonadal development. This research provides new insights into the environmental endocrine eco-toxicological effect on fish and arouses concern for potential risks of MT to aquatic organisms.

**Keywords:** 17 $\alpha$ -Methyltestosterone; *Gobiocypris rarus*; Brain; Transcriptome

## The Mechanisms Involved in Byssogenesis in *Pteria Penguin* Under Different Temperatures

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**Abstract:** Byssus is important for marine bivalves to adhere robustly to diverse substrates and resist environmental impacts. The winged pearl oyster, *Pteria penguin*, can reattach or not reattach to the same environment, which leaves the development and survival of the oyster population at risk. In this study, diverse methods were employed to evaluate the byssus quality and explore the mechanism of byssus secretion at different temperatures. The results demonstrated that oysters maintained their byssus properties at different temperatures through polyphenol oxidase (PPO) and reactive oxygen species (ROS) variation. They were both higher at 27 °C than at 21 °C. Furthermore, PPO activities of WB27 (31.78 U/g ± 1.50 U/g) were significantly higher than NB27, WB21, and NB21. Sectional observation revealed three types of vesicles, from which a novel vesicle might participate in byssogenesis as a putative metal storage particle. Moreover, cytoskeletal proteins may cooperate with cilia to transport byssal proteins, which then facilitate byssus formation under the regulation of upstream signals. Transcriptome analysis demonstrated that protein quality control, ubiquitin-mediated proteolysis, and cytoskeletal reorganization-related genes contributed to adaptation to temperature changes and byssus fabrication, and protection-related genes play a critical role in byssogenesis, byssus toughness, and durability. These results were utilized to create a byssogenesis mechanism model, to reveal the foot gland and vesicle types of *P. penguin* and provide new insights into adaptation to temperature changes and byssus fabrication in sessile bivalves.

**Keywords:** *Pteria penguin*; Byssus fabrication; Foot structure; Transcriptome; Elevated temperature

## Roles of *rpoN* in biofilm formation of *Vibrio alginolyticus* HN08155 at different cell densities

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**Abstract:** *RpoN* ( $\delta 54$ ) as a global regulator controls crucial virulence-associated phenotype, which can regulate flagellum and exopolysaccharides (EPS) during pathogenic biofilm formation. However, the knowledge of the roles of *rpoN* in biofilm formation of *V. alginolyticus* is limited, especially at different cell densities. Herein, deletion mutant strain  $\Delta rpoN$ , complementary strain  $\Delta rpoN$ -C and negative control strain  $\Delta rpoN$ -Z were constructed to investigate the effects of *rpoN* on biofilm formation of *V. alginolyticus* HN08155 based on flagellum and EPS at different cell density conditions. The results showed that all of strains can form biofilm, and biofilms of strains with *rpoN* were formed at low cell density (LCD) and detached at high cell density (HCD), while those of  $\Delta rpoN$  and  $\Delta rpoN$ -Z were absent at LCD and accumulated excessively with a spotty pellicle at HCD without detaching. The EPS contents of strains with *rpoN* was greater than that of  $\Delta rpoN$  and  $\Delta rpoN$ -Z at LCD, while the opposite trends were observed at HCD. The expression levels of *rpoN* were quantified, which were consistent with the trend of biofilm formation. It's worth noting that absence of *rpoN* resulted in the failure of biofilm detachment, lacking of flagellum and decreasing motility, indicating that *rpoN* was not necessary for biofilm formation, but it was essential for biofilm detachment.

**Keywords:** *Vibrio alginolyticus*; *rpoN*; biofilm formation; biofilm detachment; cell density; gene expression

## Food Safety Status and Veterinary Medicine Inputs of Marine Cage Culture in China

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**Abstract:** China has the highest aquaculture production in the world, and marine cage culture is one of the most important aquaculture systems. The large yellow croaker farmed in marine cages is the most important finfish mariculture species in China. Food safety of aquaculture products is an important part of the sustainable development of aquaculture. Along with industrialization and increased income levels, consumers in China pay more attention to food safety issues, and aquaculture needs to improve the food safety of aquatic products. The present study analyzed food safety status and veterinary medicine inputs of marine cage culture in China. A total of 5,000 food safety testing results of cage-cultured products, including large yellow croaker, groupers, and golden pomfret, were collected and analyzed. The qualification rate of cage-cultured products was around 97%, and the use of antibiotics was the main source of food safety problems. In order to find out veterinary medicine inputs of marine cage culture in China, we surveyed 120 large yellow croaker farms and eight drug stores in the main farming areas Fujian and Zhejiang. The survey found that 86% of farmers used veterinary medicines, and enrofloxacin is the most used veterinary medicine and had the highest sales, which is consistent with the food safety testing results. We suggest improving the food safety level of China's marine cage culture products by strengthening supervision, improving farming techniques, and establishing certification and traceability systems.

**Keywords:** cage aquaculture; China; food safety; Large Yellow Croaker; Veterinary drug residues

## Large-scale Fermentation of *Lactobacillus pentosus* 292 for the Production of Lactic Acid and the Storage Strategy Based on Molasses as a Preservative

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**Abstract:** Given the advantages of *Lactococcus pentosus* 292 as a member of lactic acid bacteria (LAB) in the high capability of producing lactic acid (LA) and excellent probiotic efficacy for shrimp aquaculture, a cheap medium containing 30 g/L glucose, 15 g/L yeast powder, 8 g/L K<sub>2</sub>HPO<sub>4</sub>, and 10 g/L tween-80 was developed for this strain, and the LA produced in this medium was up to 16.24 g/L increased by 83.48% compared with that in MRS medium after 24 h fermentation under optimal condition (temperature 35 °C, pH 6, shaking speed 100 rpm, and inoculation amount 3%). Subsequently, the large-scale fermentation kinetics models of bacterial growth (OD<sub>600nm</sub> and dry weight), substrate consumption (total sugar and reducing sugar), and product generation (LA and total protein) in a 200-L fermenter were built using better-fitting models of Logistic, Luedeking, and Luedeking-Piret, respectively. The results indicated both substrate consumption and product generation were closely related to bacterial growth. Then, a fermentation broth storage strategy based on 1-3% molasses as a preservative was developed, which besides maintaining the fermentation broth at a low-pH environment, also could effectively extend the survival period of bacteria. The storage period model established by a temperature-accelerated test showed that the 292-strain fermentation broth could be preserved for 36 days at 4 °C and 16 days at 25 °C, thereby gaining time for post-processing of fermentation broth for the preparations of cells and products without destroying activity. This work besides constructing the large-scale fermentation kinetics models of *L. pentosus* 292, also developed a storage strategy based on the molasses as a preservative, thereby facilitating the application of 292-strain for large-scale production of LAB preparation and its products.

**Keywords:** lactic acid bacteria; lactic acid; large-scale fermentation kinetics model; storage strategy; *Lactobacillus pentosus*.

## Development of RT-ERA and LFD-ERA Detection Methods for Group Enteric Microsporidian (*Enterospora epinepheli* sp. n.)

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**Abstract:** Groupers are the marine finfish belonging to the subfamily Epinephelinae and they are widely distributed in the tropical and subtropical coastal waters, with high nutritional and economic values. They are susceptible to enteric microsporidian (*Enterospora epinepheli* sp. n.) infection characterized by anorexia, weight loss, white feces and less intestinal content, and finally in severe mortality. This poses a substantial threat to the health and economic stability of the grouper fish industry. Therefore, the development of a rapid, efficient and accurate clinical test method is crucial for the prevention and treatment of enteric microsporidian infection. Enzymatic recombinase amplification (enzymatic recombinase amplification, ERA) is a nucleic acid amplification technology without large instruments requirement, that can quickly and accurately detect pathogens at a constant temperature. In this study, enteric microsporidian infected pearl gentian grouper (*Epinephelus lanceolatus*♂)× (*Epinephelus fuscoguttatus*♀) was used as materials, and specific primers of the conserved gene 18s RNA of enteric microsporidian were designed to develop real-time quantitative ERA (RT-ERA) and lateral flow dipstick ERA (LFD-ERA) detection methods. The results of RT-ERA reaction LFD-ERA can be obtained in 12 minutes and 5 minutes respectively; In the specificity test, only enteric microsporidian positive can be detected in RT-ERA reaction and LFD-ERA reaction, while those reactions with the common grouper pathogens *Amyloodinium ocellatum*, *Cryptocaryon irritans*, *Vibrio alginolyticus*, *V. harveyi*, *Vibrio Parahaemolyticus*, *Photobacterium damsela*, *Streptococcus iniae*, *viral nervous necrosis*, *Singapore grouper iridovirus* without cross-reaction, demonstrating a good specificity; In the sensitivity test, the lowest limit of detection for the reaction between RT-ERA and LFD-ERA was both 10<sup>1</sup> copies/reaction, and the sensitivity is about 100 times higher than the conventional PCR; The RT-ERA assay results show the good repeatability and stability. In conclusion, the developed ERA detection methods for grouper enteric microsporidian have the advantages of simple and fast, good specificity and high sensitivity. This research provides an important basis for the prevention and control of enteric microsporidian infection, and has good application value. It can also provide new ideas for the detection conditions lacking specialized large instruments.

**Keywords:** Group; Enteric microsporidian; Enzymatic recombinase amplification (ERA); RT-ERA; LFD-ERA

## **Antioxidant Capacity and Composition Analysis of Metabolites of *Alteromonas macleodii* QZ9-9: the Possibility of High Value-added Utilization by Macroalgae**

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**Abstract:** Agar accounts for ~60% of the dry weight of macroalgae, so the resource utilization of agar is of great significance to improve the added value of macroalgae. Herein, *Alteromonas macleodii* QZ9-9 isolated from tropical *Gracilaria* in Hainan Island was characterized as an agarolytic bacterium with relatively high agar-degrading activity. The highest diameters of the degradation circles of the *A. macleodii* QZ9-9 and its agarase of 12.16 U/mL were 41.46 mm and 22.89 mm, respectively. Additionally, the first-order degradation rate constants of this strain and its agarase were 0.02 h<sup>-1</sup> and 0.77 U<sup>-1</sup>, respectively. Importantly, the fermentation products of *A. macleodii* QZ9-9 exhibited antioxidant activity and the peak of DPPH scavenging activity of strain QZ9-9 fermentation products after culture for 50 h was up to 50.79% in the reaction for 1 h, particularly the DPPH scavenging activity of small molecule metabolites ( $\leq 3$  kDa) was up to approximately 85.85%. Subsequently, a total of 766 metabolites were detected in the small molecule metabolites by metabolomics. The peptide-like metabolites, such as prolyl-histidine, isoleucyl-histidine, isoleucyl-proline and arginyl-proline, and the antioxidant maculosin were found in the top 20 metabolites with relatively high abundance, and the antioxidant activity of maculosin was verified in this work. Therefore, we reason that the small molecule metabolites of *A. macleodii* QZ9-9 with relatively high antioxidant activity are the strong candidates for preparing non-toxic antioxidants, thereby laying a foundation for facilitating the high value-added utilization of macroalgae in the fields of cosmetic, food preservation and pharmaceutical industries.

**Keywords:** agarolytic bacterium; *Alteromonas macleodii*; agarase; antioxidant peptides; maculosin

## Transcriptomic Analysis Revealed the Dynamic Response Mechanism to Acute Ammonia Exposure in the Ivory Shell, *Babylonia areolata*

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**Abstract:** The ivory shell (*Babylonia areolata*) is an economically important shellfish in tropical and subtropical regions, but its intensive culture and biological characteristic of hiding in the sandy substrate make it highly susceptible to ammonia stress. In this study, we investigated the dynamic changes in histopathology, oxidative stress, and transcriptome of the ivory shell at different time points under high concentration (60 mg/L) ammonia exposure. With prolonged exposure to stress, vacuoles appeared in the hepatopancreas while cell volume and intercellular space increased. The activities of superoxide dismutase (SOD) and catalase (CAT) decreased significantly under high concentrations of ammonia-induced stress while malondialdehyde (MDA) levels increased significantly. Integrated analysis of differentially expressed genes (DEGs), weighted gene co-expression network analysis (WGCNA), and quantitative real-time polymerase chain reaction (qRT-PCR) revealed that lipid transport primarily contributed to maintaining cellular homeostasis during the early stage of stress (6 and 12 h). Subsequently, a significant upregulation of oxidation-reduction reactions occurred at the middle stage (24 h), leading to oxidative stress. Finally, during the later stage (48 h), metabolic decomposition provided energy for survival maintenance. Additionally, lysosome and apoptosis were identified as potential key pathways in response to acute ammonia toxicity. Overall, our findings suggest that ivory shells can respond to acute ammonia toxicity via immune and antioxidant defense mechanisms but sustained high concentrations may cause irreversible damage. This study provides valuable insights into the response mechanism of mollusks towards ammonia and serves as a data reference for breeding ammonia-tolerant varieties of ivory shells.

**Keyword:** *Babylonia areolata*; acute ammonia stress; transcriptome; histopathology; oxidative stress

## Characterization of Bacterial Communities on the Surface of *Trachinotus ovatus* fish during *Cryptocaryon irritans* Infection

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**Abstract:** *Cryptocaryon irritans*, a common parasitic organism in marine fish, has significant adverse effects on China's aquaculture industry. This study explores the potential role of the bacterial community in *Trachinotus ovatus* fish infected with *C. irritans*. Surface bacterial samples were collected at different time points post-infection (0 hours, 24 hours, 72 hours, 120 hours, and 168 hours). High-throughput sequencing of 16S rRNA was employed to analyze the bacterial composition. The results indicate that the bacterial community is mainly composed of Proteobacteria, Actinobacteria, and Bacteroidetes, with Proteobacteria consistently dominant. Following infection, there is an increase in the relative abundance of *Nautella*, *Vibrio*, and *Pseudomonas*. Alpha diversity analysis reveals a significant decrease in richness and diversity immediately after infection ( $P < 0.05$ ), followed by a notable increase post-infection clearance ( $P < 0.05$ ).

**Keywords:** Bacterial; *Trachinotus ovatus* fish; *Cryptocaryon irritans*;

## Study on *flgJ* Gene Function of *Vibrio alginolyticus*

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**Abstract:** *Vibrio alginolyticus*, as a major Marine pathogenic vibrio, has brought great harm to aquaculture industry. As a pathogenic factor, flagella plays an important role in the process of invasion of the host. The structure of flagella requires a variety of proteins, such as FlgJ, to maintain its normal function. In a pathogenic *Vibrio* HN08155 strain isolated from diseased grouper in the laboratory, it was found that the size of two FlgJ proteins and the length of base sequence were also very different. In this paper, we constructed *flgJ* deletion strains  $\Delta flgJ_1$  and  $\Delta flgJ_2$  of *Vibrio alginolyticus* flagella-related gene *flgJ*, and compared the functional similarities and differences of the two *flgJ* genes after FlgJ deletion and the formation of flagella and biofilm by dynamic test and biofilm formation ability observation. The results showed that the colony diameters of  $\Delta flgJ_1$  and  $\Delta flgJ_2$  were smaller than those of the original strains, indicating that the colony migration ability of the two missing strains was weakened and the movement ability of the bacteria was decreased. When observing biofilm formation, it was found that the biofilm growth ability of  $\Delta flgJ_1$  was not good at 6 hours, and the biofilm adhesion ability was decreased, while the biofilm formation amount was increased and polysaccharide expression was increased at 24 hours. Based on the results of this study, it can lay a certain foundation for the comprehensive understanding of the function and characteristics of *Vibrio alginolyticus flgJ* gene and the prevention and control of vibrio biofilm in mariculture environment.

**Keywords:** *Vibrio alginolyticus*; *flgJ*; biofilm formation; flagellum

## Characteristics and risk assessment of PFASs pollution in surface water, sediment and wild fish in key basins of Hainan Island.

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**Abstract:** PFASs is a kind of artificial persistent organic pollutants, widely existing in various environmental media and organisms, which has environmental persistence, bioenrichment and biotoxicity, and can destroy the biological environment and human health. The Nandu, Changhua and Wanquan rivers are the three major rivers of Hainan Island. They are the habitats of biodiversity and their dependence, feeding tens of millions of people along their banks. However, the study on PFASs in the key watershed of Hainan Island has not been reported so far. Therefore, this study investigated the pollution concentration characteristics, spatial distribution, potential sources, occurrence characteristics of each fish species, biological enrichment and human health exposure risk assessment of 20 PFASs species in surface water, sediments and wild fish in three major rivers of Hainan Island. The results showed that the average concentration of PFASs in surface water was 7.49 ng/L, and the concentration range was 6.62-8.74 ng/L, mainly PFBA and PFOA. The average concentration of PFASs in sediments was 0.90 ng/g dw, and the concentration ranged from 0.28 to 2.59 ng/g dw, mainly PFUnDA and PFPeA. The average concentration of PFASs in wild fish was 61.13 ng/g dw, and the concentration range was 15.08-197.92 ng/g dw, mainly PFHxA and PFBA. The presence and spatial distribution of PFASs detected in surface water and wild fish between upstream and downstream are evident, with the continued input of existing PFASs point sources in large rivers on Hainan Island. PFASs pollution in the lower reaches of surface water and wild fish is more serious, and PFOS and PFOA pollution exist in the lower reaches of Changhua River and Wanquan River respectively. We also found that there may be potential health risks if long-term consumption of *Opsariichthys bidens* in the lower reaches of the Changhua River. Different species, habitat environment, feeding behavior and nutrient level of fish will lead to differences in the concentration of PFOS. The spatial variation trend of PFASs in large rivers in Hainan Island is influenced by the domestic and industrial sewage discharge of coastal cities, the dilution and diffusion degree of water bodies, and the urban GDP, population density, industrial type and hydrodynamic conditions. The results of this study will provide data support and reference for the prevention and control of PFASs in key river basins of Hainan Island.

**Keywords:** PFASs; Surface water; Sediment; Wild fish; Risk assessment

## **Effect of Temperature and Phosphate Levels on the Growth and Photosynthetic Activity of *Isochrysis galbana***

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**Abstract:** Ocean warming and shallowing of the mixed layer will affect nutrient availability, leading to intricate consequences for marine phytoplankton. However, there is limited understanding regarding the adaptive response of *Isochrysis galbana* to ocean warming and phosphate fluctuations. To address this knowledge gap, we conducted simulated culture to examine the physiological response and adaptation mechanisms of *I. galbana* to concurrent changes in temperature and phosphate levels. The cultures were subjected to four different treatments, consisting two temperatures conditions (LT: 25°C, HT: 29°C) and two phosphate concentrations (LP: 2.75 μmol/ L, HP: 7.2 μmol/ L). The results indicated that when phosphorus was limited (LP condition), elevated temperature did not have a significant impact on the growth and maximum quantum yield (Fv/Fm) of *I. galbana*, but did result in a reduction in the photosynthetic activity. Conversely, when phosphorus was abundant (HN condition), high temperature did not alter the growth or Fv/Fm. The levels of chlorophyll a and carotenoids exhibited a similar pattern, decreasing and then increasing in both conditions. The alkaline phosphatase (AKP) activity observed in the phosphorus limited group (LP) exhibited a significant increase, indicating that *I. galbana* has the ability to utilize organic phosphorus in order to acquire inorganic phosphorus through the upregulation of AKP activity, this mechanism serves to mitigate the adverse effect of low phosphorus stress and sustain growth. The present study enhances our understanding of the species-specific adaptation mechanism of *I. galbana* to future oceanic alterations.

**Keyword:** Ocean warming; Phosphate; *Isochrysis galbana*; Transcriptome

## Study on the Structural Characteristics of Tropical Seaweed Attached Biomes

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**Abstract:** Seaweed and surface attached microbial communities can be regarded as symbiotic organic wholes which plays key role in growth and development. However, there is a lack of understanding of the relationship between seaweed and attached organisms. It is very important to study the differences of biodiversity and core species of attached microbial communities during the growth of different seaweeds. In the present study, 16S rRNA gene amplification sequencing technology was used to analyze the bacterial community diversity and core groups of *Sargassum polycystum* and *Eucheuma gelatinae*, compare the changes of bacterial communities during the growth of *S.polycystum* and *E.gelatinae*, analyze the physical and chemical indexes of seawater and illustrate the environmental factors affecting the composition of attached microbial community. The results showed that *Proteobacteria*, *Bacteroidota* and *Cyanobacteria* were the dominant groups of *S.polycystum* and *E.gelatinae*, and the common species accounted for 42.68%. The *Proteobacteria* OTUs accounted for 75% of all samples. There was no significant difference in alpha diversity between the attached bacterial communities of *S.polycystum* and *E.gelatinae* ( $p>0.05$ ). The species composition of bacterial community between *S.polycystum* and *E.gelatinae* was different according to PCA analysis. The relative abundance of *Fusobacteriota*, *Dadabacteria*, *Planctomycetota*, *Desulfobacterota* and *Myxococcota* were significantly different between *S.polycystum* and *E.gelatinae* ( $p>0.05$ ). The total relative abundance of *S. polycystum* and *E. gelatinae* core OTUs increased over time. The *Chl-a* concentration increased in growing environments of *S.polycystum* and *E.gelatinae*, while the TN concentration decreased. The relative abundance of *Proteobacteria*, *Bacteroidota* and *Cyanobacteria* was positively correlated with *Chl-a*, but negatively correlated with TN. This study provides a vital baseline knowledge on the attached microbial community composition of *S. polycystum* and *E. gelatinae*, and provides a scientific basis for the management of offshore seaweed resources and the sustainable development of seaweed cultivation in Hainan Island.

**Keyword:** Seaweed; Microbial community; Attached biomes; Diversity; 16S rRNA

## Study on the Artificial Infection Conditions Of *Branchiomyces* on Crucian Carp

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**Abstract:** Exploring the conditions of *Carassius auratus* infected by *branchiomyces* for establishing a fish gill mold infection model and further screen effective drugs against fish gill mold disease. Under two different nutrient conditions (PDA medium and FX medium), The two-factor three-level random block experiment method was applied to optimize the environmental factors of germination for *branchiomyces* spores. Take Crucian carp species as the subject, By means of microscopic observation of pathological changes, The infection conditions of were studied from two aspects of wound pattern of Gill and the infection method of *branchiomyces* spores. The best germination environment factor is the culture temperature 20°C, pH=8.0. Through abrasion, freezing, and drug-induced wounds, unilateral gill injuries were inflicted on *Carassius auratus*. Compared with the control group, observeing with the naked eye that CK and T groups' gills stick to Operculum. The gill filaments of group T2 were white in large areas. Examining the gills through stereoscope, The gill filaments of CK Group were hyperemia, and the gill edges of T group were Hyperemia with lots of Mucus. Compared with the control, the gill cartilage and Gill filaments of group CK were full of dendritic black filaments, and the gill filaments of group T soaked in gill mold spores for a long time, the gill filaments were black, and the branch-like black filaments could be observed on the gill filaments of T1 and T3 groups compared with T2 groups. This study determined the optimal conditions for the production and germination of *branchiomyces* spores , and the conditions for *branchiomyces* infection initially laid a foundation for the establishment of branchial mould infection model.

**Keywords:** *Branchiomyces*; *Carassius carp*; Conditions for sporulation; Artificial infection

## **Effect of Purification Cultivation Duration on the Survival and Nutritional Profile of oyster *Crassostrea angulata* in Sanya Bay, Hainan, P. R. China**

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**Abstract:** The present study investigates the impact of different purification cultivation durations on the survival and nutritional composition of oysters (*Crassostrea angulata*) cultivated in Sanya Bay, Hainan, People's Republic of China. The aquaculture industry is increasingly focusing on the optimization of cultivation practices to enhance the quality of harvested shellfish. In this context, the purification cultivation duration is considered a critical factor influencing both the survival rates and nutritional attributes of oysters. A series of experiments were conducted over varying purification cultivation periods, ranging from short to extended durations, to elucidate the optimal conditions for oyster growth and health. Survival rates were monitored, and nutritional analyses were performed to evaluate the protein, lipid, moisture and carbohydrate of the oysters. The purification lasted for 75 days, starting from April 2023 and ending in July 2023. The survival rate of oysters ultimately reached 93.94% (a total of 1280, with 82 deaths) until the experiment finished. Furthermore, the results indicate that the content of crude fat and ash were trended to change significantly during the purification cultivation stage ( $P < 0.05$ ), while the moisture, crude protein content, and fatness were slightly changed without significant difference ( $P > 0.05$ ). Extended purification cultivation periods appear to positively correlate with improved survival rates, potentially indicating enhanced resistance to environmental stressors. Additionally, longer cultivation durations may contribute to alterations in nutritional composition, emphasizing the need for a balanced approach that considers both survival and nutritional quality. This study contributes valuable insights into the optimization of purification cultivation practices for *Crassostrea angulata*, providing a foundation for sustainable and high-quality oyster production in Sanya Bay, Hainan, P. R. China. The results may have implications for aquaculture management strategies, offering guidance for practitioners seeking to maximize both the survival and nutritional benefits of cultivated oysters.

**Keywords:** *Crassostrea angulata*; purification; nutritional Profile

## Development of a Non-destructive Technique for the Detection of Neural Necrosis Virus in Grouper

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**Abstract:** *Plectropomus leopardus*, commonly known as the *Eastern star-spotted* or *leopard gill spiny perch*, is a warm-water reef fish with a primary distribution in the Western Pacific Ocean extending to the Indian Ocean and other tropical waters. Its presence is notable around Hainan Island and the South China Sea islands and reefs. This species holds significance as a valuable seawater economic fish due to its delectable and nutritious meat, garnering favor among consumers and presenting a promising market outlook. The escalating market demand has spurred the rapid growth of Eastern star-spotted aquaculture, establishing it as a popular species in Hainan Province. However, the intensification of culture density and the degradation of the culture environment have given rise to various culture-related diseases, significantly impeding the sustainable development of Eastern star-spotted aquaculture. Through practical aquaculture experiences, it has been identified that the primary viral disease affecting Eastern star-spotted is the *Nervous Necrosis Virus* (NNV). This virus can be transmitted vertically through fertilized eggs and horizontally through water, bait, and feces, leading to severe infections in young fish and juveniles. In extreme cases, mortality rates of cultured fry can exceed 90% within one week. Current consensus suggests that the most effective strategy for preventing and controlling Eastern star-spotted virus disease involves screening healthy parents devoid of virus particles to interrupt vertical transmission. Additionally, enhancing the sensitivity of virus particle detection in the water environment is crucial for inhibiting horizontal transmission. Despite this, there is a dearth of efficient early screening techniques for Eastern star-spotted virus during the actual culture process. Existing virus detection methods often rely on isolating the virus from infected live tissue samples, a procedure that poses inherent drawbacks, including varying degrees of damage and, in some instances, fatal consequences for the fish. Consequently, there is an urgent need to develop a rapid and efficient screening technique for Eastern star-spotted virus that does not compromise the well-being of the fish.

**Keywords:** *Eastern star-spotted*; *Nervous necrosis virus*; aquaculture; viral copy number; Virus detection technology

## Analysis Of The Effect Of Acetylation Modification Of Lactate Dehydrogenase On Its Function In *Phaeodactylum Tricornutum*

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**Abstract:** Acetylation and acylation post-translational modification widespread in almost all organisms. Acetylation and acylation have many connection nodes. However, it is not clear how they are linked in eukaryotic microalgae. In order to study the relationship between acetylation and acylation in eukaryotic microalgae. This study starts with LDH of Phaeophyta *Tricornutum*, LDH gene was amplified by PCR. The Lys220 was mutated to Arg and Glu by site-directed mutagenesis to simulate acetylation and deacetylation modification. The LDH gene and its mutants were ligated into the prokaryotic expression vector pMBP-C by double digestion and homologous recombination, and the clone identified correctly by digestion and sequencing was transformed into *E. coli* BL21. The protein expression and its mutants was induced by IPTG, and the recombinant protein separated and purified using Ni-NTA spin columns Ni-affinity chromatography followed by western blot for positive identification. The results showed that the induction by 0.02 mM IPTG, the recombinant protein was purified at the optimized temperature of 4 °C for 24 h and eluted with 250 mM imidazole for purification. The western blot confirmed the protein weight to be as expected. The recombinant proteins of LDH and its mutants were successfully obtained, which provided a basis for the subsequent study of the effect of LDH acetylation on protein activity and the interaction between acetylation and acylation in eukaryotic microalgae.

**Keywords:** *Phaeodactylum tricornutum*; Acetylation; LDH; site-directed mutation

## Spatiotemporal Variation of Methane Emissions in Sansha Bay, Fujian Province: Insights from Long-Term Monitoring and Microbial Community Analysis

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**Abstract:** Methane (CH<sub>4</sub>), a pivotal greenhouse gas with a global warming potential 20 times greater than carbon dioxide, plays a significant role in the greenhouse effect. This study focuses on Sansha Bay, situated on the northeastern coast of Fujian Province, renowned as a vital breeding ground for marine organisms. Through extensive tracking and monitoring of methane emissions in this breeding area, our investigation reveals notable findings: (1) Dissolved CH<sub>4</sub> in the surface seawater of Sansha Bay serves as a source for atmospheric CH<sub>4</sub>; (2) Significant spatial and temporal variations exist in the concentration and saturation distribution of dissolved CH<sub>4</sub>, with elevated values observed during summer and in areas influenced by industrial activities. Further exploration utilizing DNA/RNA high-throughput sequencing technology identifies Proteobacteria and Actinobacteria as predominant bacterial communities in the region. Although methanogenic bacteria like *Clostridium* and *Rumiclostridium* exhibit low abundance, they display heightened metabolic activity, particularly in the estuary breeding area and central restricted zone. Additionally, statistical analyses reveal a significant correlation between bacterial taxa and methane emissions in Sansha Bay. This research contributes to a comprehensive understanding of spatial and temporal changes in greenhouse gas (CH<sub>4</sub>) emissions within breeding areas and emphasizes the impact of bacterial communities on methane dynamics.

**Keywords:** Methane emissions; microbial community analysis; DNA/RNA high-throughput sequencing; spatial and temporal variation.

## Spatio-temporal Dynamics of Carbonate System from Sansha Bay, Southeast China

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**Abstract:** The seawater carbonate system plays an important role in the global carbon cycle. With prominent problems such as global climate change, the seawater carbonate system has also changed, especially in the coastal waters, due to human activities and surface runoff. Here, dissolved inorganic carbon (DIC), total alkalinity (TA) and surface seawater  $p\text{CO}_2$  were measured monthly in different mariculture zones and non-farmed areas from Sansha Bay, a typical aquacultural bay in southeast China. Mean surface seawater TA and DIC ranged from 610.50 to 2344.05  $\mu\text{mol/L}$  and 485.93 to 2273.80  $\mu\text{mol/L}$ , respectively. TA generally showed a downward trend from January to May and a slight rebound in June both in farming areas and non-farmed areas, except for algae farming zone where the region has remained stable. Algae farming area is always the highest region of TA, while non-farmed area is the lowest with wide ranges. However, there is no significant difference in DIC dynamics among the cultivation of algae and the fish or shellfish aquaculture. It suggests that the cultivation of macroalgae plays an important role in maintaining the total alkalinity, but has little impact on DIC. For algae farming area, the surface seawater  $p\text{CO}_2$  was highest in January, followed by June and May, ranging from 213.24  $\mu\text{atm}$  to 2039.25  $\mu\text{atm}$ . With the growth of macroalgae,  $p\text{CO}_2$  remained a low level. It showed negative sea-air  $p\text{CO}_2$  from March to May, suggesting the potential for carbon sink. For shellfish farming areas, the fluctuation of surface seawater  $p\text{CO}_2$  is very small and low. In non-farmed zones,  $p\text{CO}_2$  fluctuations are the largest and peak in May to June, which is significantly different from farmed zones. Finally, we found that salinity was the most direct factor affecting the carbonate systems of seawater. The main factor affecting salinity in the bay is precipitation, because there is no significant difference between estuary and other areas on dry days, but a significant difference is detected after precipitation. Meanwhile, the influence of human activities including shipping and offshore industry on the carbonate system is relatively small.

**Keywords:** Fishery carbon sink; carbonate system; environmental factors; spatial-temporal dynamics; mariculture

## Investigation of Zooplankton Community in Luoma Lake

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**Abstract:** Understanding the composition, diversity, and abundance of zooplankton communities is crucial for better utilization of Luoma Lake, as zooplankton are the second link in the aquatic system's food chain (they are also excellent biological indicators of aquatic health as they are located at the center of the food web), and Luoma Lake. Most of the physicochemical parameter values recorded in this study are within the standard range required by the zooplankton community. Among the 21 species, the first, second, and lowest records were recorded for the hook *Pleuroxus hamulatus*, *Leptodora kindti*, and *Ceriodaphnia cornuta*, respectively. There is currently no relevant literature on the investigation of zooplankton communities in Luoma Lake. This study has laid a certain foundation for the food web of Luoma Lake. In order to protect the fish diversity of Luoma Lake, it is recommended to strengthen the investigation of zooplankton and further optimize the ecological structure.

**Keywords:** Luoma Lake; Zooplankton community

## **Session 6 Economics, Gender, Livelihood and Policies**

### **专题六：经济性别生计与政策**

## **An Assessment of the Impact of RCEP Implementation on Intra-Member Aquatic Product Trade and its Influencing Factors**

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**Abstract:** In the current context of the inefficiency and dysfunction of the World Trade Organization (WTO), coupled with a slowdown in global economic growth and the persistent rise of trade unilateralism and protectionism, the implementation of the Regional Comprehensive Economic Partnership (RCEP) offers a new avenue for trade and economic growth in the Asia-Pacific region. Trade in aquatic products, as a significant component of agricultural trade, holds a crucial position in Asia-Pacific. In light of this, this study examines the potential impact of RCEP's implementation on aquatic product trade among the contracting parties, focusing on both tariff recessions and non-tariff measures. The results indicate that RCEP's tariff recession efforts are modest among most RCEP countries compared with the existed FTAs, but it will significantly lower the aquatic product tariff barriers among China, Japan and Republic of Korea, which had not previously signed FTAs with each other. In terms of non-tariff measures, RCEP exhibits certain progress compared to other FTAs in its provisions regarding rules of origin, Sanitary and Phytosanitary (SPS) measures, Technical Barriers to Trade (TBT), and dispute resolution procedures etc.

**Keywords:** RCEP Aquatic product trade; Tariff concession; Non-tariff measures

## The Sustainable Development of the Aquaculture Sector

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**Abstract:** Fish constitutes about 75% of the animal protein intake for the Cambodian households and most of it comes mainly from freshwater fisheries in both fresh and processing form. Besides inland fisheries, aquaculture contributes around 10% of the total inland fish catch and plays an important role and is considered to have great potential for augmenting fish production in Cambodia. Fisheries and aquaculture is believed to have enormous potential to provide the poor people with more food, better nutrition and increased incomes. They also stimulate economic growth and offer greater diversification of their livelihoods. Within the freshwater aquaculture model in Cambodia, cage culture represents the highest percentage of about 70% of aquaculture production while pond culture covers only 30%. The most important and high profit fish species in cage culture system in Cambodia is Trey Chhdaur (Giant Snakehead, *Channa Micropeltes*). As originated in Cambodia, cage culture has increased and being operated in the Mekong Basin, including the Tonle Sap Great Lake (42%), the Tonle Sap River (17%), the upper stretch of the Mekong River (19%), lower stretch of the Mekong River (14%) and Bassac River (7%) (So et al., 2005). For several decades, fish products from cage culture were sold to domestic market in both fresh and processed forms. Aquaculture has only relatively recently become the focus of sustained interest from research and development institutions in Cambodia. This interest aligns with predicted, and increasingly realized, declines in inland capture fisheries production. For example, a combination of drought and water impoundment by upstream dams caused reported fish catch from the Tonle Sap to contract 23% in 2020, prompting fears of imminent fisheries collapse. Such a collapse would threaten the livelihoods and food security of millions of Cambodians. The rapid development of aquaculture sector has led to economically unstable conditions. The farming systems have been spontaneous and mainly uses live feed such as fresh trash fish, marine fish, yellow apple snails that cause environmental pollution and the depletion of natural aquatic resources in freshwater. The price of commercial cultured fish is not stable because they are strongly affected by the seasonal supply of wild captured fish as well as seasonality in aquaculture farming while they are mainly used for domestic consumption. Some critical challenges facing the aquaculture sector in Cambodia include: (1) Overfishing: Overfishing is a major problem in Cambodia. This is leading to a decline in fish stocks, which is threatening the sustainability of the aquaculture sector, (2) Pollution: Pollution from agricultural runoff and industrial waste is a major problem in Cambodia. This is harming fish populations and making it difficult to produce high-quality fish, and (3) Disease: Disease is a major problem in the aquaculture sector. This is leading to high mortality rates and reducing productivity. However, with the aquaculture sector, there are a range of opportunities available for investment in this sector include: (1) Large-scale commercial operations: As mentioned, there is a growing trend towards larger-scale commercial aquaculture operations in Cambodia by using modern technologies and equipment, and exporting

their products to international markets, (2) Fish feed production: The production of fish feed is a critical component of the aquaculture industry, and there is currently a shortage of high-quality fish feed in Cambodia. Investors with experience in this area could find significant opportunities to supply the industry with the feed it needs, (3) Fish processing and value-added products: With the demand for seafood continuing to grow globally, there is increasing interest in value-added products such as fillets, smoked fish, and seafood snacks. Investors with experience in fish processing and marketing could find opportunities to develop these types of products for export markets, and (4) Technology and equipment: The aquaculture industry in Cambodia is still largely dominated by traditional, small-scale farmers. Investors with experience in aquaculture technology and equipment could find opportunities to provide these farmers with the tools and resources they need to modernize their operations. The government has announced plans to invest \$100 million in the aquaculture sector over the next five years. This investment will be used to improve infrastructure, promote research and development, and support the growth of the sector. The aquaculture sector is expected to grow by 10% per year over the next five years. This growth will be driven by rising demand for fish, both domestically and internationally. The aquaculture sector is expected to create 100,000 new jobs over the next five years. The total economic value of the aquaculture sector in Cambodia was \$200 million in 2022. This is expected to grow to \$500 million by 2027. The aquaculture sector in Cambodia is made up of a diverse range of actors, including small-scale farmers, large-scale companies, and international investors. Small-scale farmers continue to play a significant role in the industry, with the majority of fish farming still done in small, backyard ponds. These farmers typically grow a mix of fish species, including tilapia, catfish, and carp, and sell their produce in local markets. The Cambodian government has been a strong supporter of the aquaculture sector in Cambodia, recognizing its potential to drive economic growth and alleviate poverty in rural areas. In addition to the Aquaculture Development Strategy, the government has implemented a range of policies aimed at promoting the industry, such as tax incentives for aquaculture companies and subsidies for small-scale farmers to purchase fish seed and equipment. The government has also taken steps to improve the regulatory environment for the industry, with the establishment of the Aquaculture Development and Management Sub-decree in 2018. This sub-decree sets out the licensing and registration requirements for aquaculture operations, as well as the standards and guidelines for the industry.

## The Value and Application of One Community in Blue Transformation

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**Abstract:** The One Community concept endeavors to synergize aquaculture with the advancement of societal well-being through a holistic integration of technical, economic, and humane elements with the aquaculture value chain approach. This approach is ultimately aimed at achieving sustainable development. The concept places emphasis on the importance of social inclusiveness in all activities and encourages relevant agencies to embrace this integration. It also encourages stakeholders and consumers to build the aquaculture value chain as productive, sustainable, eco-friendly, and enjoyable. Key issues related to the One Community concept include gender equality, the livelihoods of all stakeholders, social and environmental impacts, culture and aesthetics, corporate culture value consensus, regional and international harmonization, and concerns for public opinion. The public is an important party in this approach. Aquaesthetics, which advocate the esthetics of aquaculture and aquatics to the public, can visualize the value of One Community for catalyzing sustainable aquaculture. This includes forms, such as eco-friendly aquaculture, contributions to global restoration, aquatic welfare, ornamental aquatic organisms, aquatic tourism, aqua culture, arts and multimedia, aquatic gastronomy, aquatic healthcare, and integrated aquaesthetics. The One Community concept helps to achieve balance between social outcomes and sustainability, which is the key goal of the FAO's Blue Transformation initiative. An example of the One Community concept in action is crayfish farming in China. The development of this industry is culturally driven, as crayfish were considered an alien ecological disaster species and treated as pests until the end of the last century. However, due to its red color and special culinary taste, the species was increasingly accepted by the public, leading to the development of large-scale farming technology around 2000-2005. Crayfish farming has grown exponentially, with an average annual growth rate of 28% since 2006, and it has rapidly surpassed the farmed production of *Penaeus vannamei* in 2018, becoming the largest farmed crustacean in China. Crayfish festivals have become brand activities of local aquatic delicacies, aquatic tourism, folk customs, culture, entertainment, and trade, attracting millions of consumers every year. The total value of the crayfish industry reached 65 billion USD in 2021, with a year-on-year increase of 22.43%. The ratio of crayfish primary (farming), secondary (processing), and tertiary value (catering) in 2021 was about 2:1:7, with a year-on-year increase of 10%, 23%, and 36%. One Community actually has been applied widely in the world, e.g., the globalization of Norwegian salmon, Japanese sashimi and sushi, and koi culture. The Network of Aquaculture Centres in Asia-Pacific (NACA) welcomes collaboration with institutions, organizations, media, and other partners on the One Community concept.

**Keywords:** One Community; aquaesthetics; value chain approach; Blue Transformation

## Aquaculture transformation in Asia and the Pacific region

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**Abstract:** Asia and the Pacific region are the global leader in aquatic food production and have the highest overall rates of consumption of these foods. Aquaculture provides 70 percent of aquatic food in the region and makes important contributions to food and nutrition security, livelihoods, economic development, and a wide range of SDGs. Asia and the Pacific region is remarkably diverse and wide-ranging geographically, in its flora and fauna, culturally, institutionally, and economically. Aquaculture is unevenly distributed in the region with highly diversified production systems. The future trajectory of aquaculture development will face different challenges than in the past, with ever-increasing impacts and constraints from climate change, water stress, limited land and coastal area, changes in international politics, and emerging trend of reversed globalization. Other persistent underlying problems include inadequate governance in the region, the lack (or unreliability) of infrastructure, unregulated development, unsustainable intensification, resource limitation, ecological and environmental constraints, science and technology deficit, value chain inefficiency, and the spread of aquatic diseases. The region farms hundreds of species in extensive, semi-intensive, and intensive culture systems, ranging in scale from small-scale backyard family fishponds to highly-industrialized, technologically-sophisticated market-driven commercial operations. Many of these aquaculture species and production systems have received little R&D investment. More than anywhere else in the world, Asia and the Pacific's need for aquaculture transformation is considerable. The future of aquaculture in the region needs transformation to secure sustainable supplies through sustainable intensification to be more labor productive, more efficient use of land and other resources, and expansion into areas not yet effectively used for food production. Aquaculture in Asia-Pacific region need to lead towards more efficient, inclusive, resilient and sustainable aquatic food systems, to reach strategic targets of improving food and nutrition security, reducing poverty and driving economic development, ensuring environmental sustainability, building resilience, and promoting inclusiveness. The priority action areas are proposed as improving technical cooperation and knowledge transfer; governance; production via efficiency, sustainable intensification and expansion; improve value chains; improve environmental performance and resilience; and social benefits. This provides a comprehensive blueprint to enable innovations and

investments for a sustainable, healthy and inclusive aquaculture future in Asia and the Pacific.

**Keywords:** Asia and the Pacific region; Aquaculture; Blue Transformation; Sustainable Intensification and Expansion; Value Chain

## What influences the intention to adopt compound feed practice? Evidence from shrimp farmer in China

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**Abstract:** With the ever-rising demand for seafood, aquaculture in China is a contributing factor to the decline in fishery stocks. Despite impressive gains, China's aquaculture sector also faces numerous challenges, sustainability is of the most questioned. For instance, marine fish is mainly used as farming feed directly, or through converting into fishmeal and fish oil indirectly. Forage fish places pressure on wild fisheries, since China's forage fish consist mainly of juveniles of commercially important species (~32 to 50%), small benthic and mesopelagic fish, crustaceans, and cephalopods. In response, in September 2003, the Ministry of Agriculture and Rural Affairs (MARA) issued the "Regulation on the Quality and Safety of Aquaculture". The regulation encourages using compound feed, restrict direct feeding of chilled miscellaneous fish, and prevent water pollution from residual bait. Then in 2020, MARA has promoted the implementation of the "Five Major Actions" for green and healthy aquaculture, including the action plan to replace the juvenile fish with compound feeds. Exploring farmers' intention to adopt compound feed technology and possible influencing factors, with standard questionnaire, we personally interviewed the shrimp farmers from six cities of five provinces in China between July and August 2022. They were Hebei province (represented by Tangshan and Cangzhou), Shandong province (represented by Rizhao), Jiangsu province (represented by Lianyungang), Fujian province (represented by Zhangzhou) and Guangdong province (represented by Zhanjiang). We find that: 1) Overall farmers have positive evaluation on innovation of compound feed. Specifically, 68.25% of the shrimp farmers expressed they will use compound feed in the future. For reasons, 62.4% of them strongly agree that compound feed improve shrimp output, 61.4% express new feed practice can reduce input cost, for instance the rapid wage growth of part-time labor (76.2%). 73.3% of farmers think compound feed improves water quality, echoing the call for green and healthy aquaculture, raised by MARD, substitute chilled miscellaneous fish with compound feed; 2) Compound feed usage depends on farm's characteristics, such as pond types (high-land pond, low-land pond, soil land pond, greenhouse pond and industrial aquaculture pond), the scale and intensity of farming operations; 3) Farmer' profile affect compound feed adoption. For instance, farmers' age, gender, educational level, farming experience and belonging to professional associations or not.

**Keywords:** feed practice; fishery sustainability; China fisheries; theory of utility maximization; innovation

## **Pilot Cage Culture at Eutrophic Coastal: A Case Study in Sriracha Bay, Chonburi Province-Thailand**

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**Abstract:** Sustainable mariculture has not been widely practiced in Thailand. The site selection, culture technique, and target species are some of the major questions. In this study, we have identified a suitable site for a test cage culture at Sriracha Bay, Chonburi Province – Thailand. The site was situated 1,000 meters from the coast and 6 meters in depth, has generally good water quality, and has a permanent online monitoring station for the hydrographic and water quality parameters, namely GOT001 station. We conducted an experimental cage culture applying the Integrated Multi Trophic Aquaculture (IMTA) technique. The culture species were selected by trophic level with sea bass as the main species and cultured with green mussel, sea cucumber, and macroalgae for reducing uneaten feed, feces or solid waste, and dissolved nutrients from the cage culture. We had successfully raised sea bass with satisfactory results over an 18-month period. However, due to severe plankton bloom occurrences in JulySeptember 2023, the sea bass was harvested. According to the result from data collected from the GOT001 station and monthly water samples analysis from stations around the cage area over 3 years, we characterized the hydrographic and water quality features at the Sriracha Bay area. Tidal mixing and currents were strong and governed mainly by tide and wind. Water quality was in good condition year-round except for the 4 months between June to midOctober. The best time for integrated aquaculture in this area was from November to June.

**Keywords:** Cage culture; Integrated Multi Trophic Aquaculture; Sriracha Bay; Sea bass

## **Shrimp Industry in China: Overview of the Trends in the Production, Imports and Exports during the Last Two Decades, Challenges, and Outlook**

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**Abstract:** China's shrimp industry has developed rapidly over the last two decades. With such a development, some major constraints to the sustainable growth of the industry have emerged. This paper aims to present an overview of China's shrimp industry over the last two decades, with special attention on farm-raised shrimp both in marine and inland waters. Extensive research on its recent evolution has been carried out based on data collected from China Fishery Statistics Yearbooks 2002 to 2023. Findings indicated that there has been an upward trend in total shrimp production in China over the last two decades, with a growth rate of 120%, representing an average annual growth rate of 4.3%. The observed higher shrimp production is driven by expanded shrimp aquaculture production, with major species dominated by the western white shrimp (*Litopenaeus vannamei*), followed by the black tiger shrimp (*Penaeus monodon*), the Chinese shrimp (*Fenneropenaeus chinensis*), and the Japanese prawn (*Penaeus japonicus*). Challenges linked to the environmental deterioration and other factors associated with farming intensification need to be properly addressed. First, shrimp farming industry should be developed in a way that promote the protection of the ecosystem and environment. Applying such an organic farming principle presupposes some practices or methods for addressing the existing issues threatening the sustainable development of the shrimp aquaculture industry. To that end, there is a need for innovative techniques that should be strengthened. Second, possible solutions to shrimp disease problems, namely prevention, early disease diagnosis, and control techniques also should be enhanced and improved, emphasis on the former. In addition, encouraging the use of good quality feed in appropriate quantity and form are also of paramount importance. It is thus worth noting that further policies need to focus on promoting large range of ecological shrimp aquaculture technologies that should be encouraged among the farmers.

**Keywords:** Shrimp aquaculture industry; Challenges; Outlook, Ecological shrimp aquaculture; China.

# **A Systematic Scoping Review of Drivers for Women Involvement in Cross-Border Fishery Trade in Sub-Saharan Region: Taking Tanzania-DRC Trade as A Case Study**

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**Abstract:** The purpose of this study is to synthesize the common drivers for women to engage in cross-border fishery trade in the East Africa and Sub-Saharan region, taking a case of Tanzania and the Democratic Republic of Congo. The study provides a spec for interventional programs and studies, as to what niceties to channel or focus when attempting to empower women in fisheries and cross-border trade. It employs a systematic scoping review using three databases of Science Direct, Web of Science, and the Google Scholar. Evidence gathered from a total of 28 studies show that, firstly the market between Tanzania and the Democratic Republic of Congo is ruled by a set of social relations and cultural norms which defines the relationship between the product, the actors and their related activities along the value chain. Second, women are driven into cross-border trade mostly by social factors such as house-keeping, their customary position and roles in the fishery value chain, marital status, and small formal or informal groups. Also, cross-border trade seems to be more beneficial than domestic market, pertinent infrastructure, and owning capital and resources. Thirdly, despite of these drivers' women faces challenges which limits their participation in trade. As seen in the study, the challenges are both social-cultural and social-economical. The authors contended that, enhancing women position in trade, depends on how the programs approach towards their decision-making ability starting at family level, society and the whole fishery value chain. The intervening programs should be multidisciplinary as the process of empowerment starts from building personal ability through education to the whole community behavior change. Some of factors to be addressed are difficult to apprehend without societal inclusion, as they go down to disconcert the ever-assumed women's position in the social economic practices of community.

**Key words:** Cross-border fishery trade; women involvement; sub-Saharan region

## **Analysis of Economic Benefits in the Whole Process of River Crab Production——Based on the Field Survey in Yangcheng Lake Town**

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**Abstract:** The big food concept put forward by General Secretary Jinping Xi points out that we should ask for protein from rivers, lakes and seas, and river crabs, as a kind of aquatic product popular with consumers, play an important role in providing high-quality protein. This paper describes the basic situation, cost and benefit status of river crab aquaculture from the perspective of economic benefits. Based on three cases in the main producing areas of river crabs, the current situation and dilemmas of river crab aquaculture are summarized and analyzed. It is found that farmers are "crossing the river by groping the stones", and there is still much room for improvement in their farming experience and management level; natural disasters and diseases increase the risk of aquaculture, and the uncertainty of aquaculture production and income increases; there is the phenomenon of substandard products in the market, and there is an urgent need for effective regulation of the trading and circulation links; the scale of the industry is huge, but the competitiveness is not strong, and the value-added of the products is not high enough. The scale of the industry is large but the competitiveness is not strong, and the added value of the products still needs to be improved. To this end, this paper proposes that government departments should increase the cultivation level of farmers; the market should strengthen the supervision, protect the local crab brand, and crack down on the phenomenon of non-compliant products flowing into the market; the meteorological department should strengthen the weather monitoring and provide timely weather information, and the enterprises can design a reasonable aquaculture insurance program to reduce the impact of natural disasters on the farmers' economic losses, so as to realize the sustainable development of river crab aquaculture industry.

**Keywords:** River crabs; Economic benefits; Rural revitalization

## Current Practices and Challenges of Large Yellow Croaker Farming in China

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**Abstract:** Large yellow croaker (*Larimichthys crocea*) is the most economically important mariculture finfish species in China, accounting for 13.89% of total marine aquaculture production by volume, making it the highest-production finfish species. In particular, large yellow croaker aquaculture in Fujian and Zhejiang provinces contributes 83.15% and 12.71% of total large yellow croaker production in China, respectively. However, issues have arisen with current large yellow croaker farming practices, including coastal eutrophication, plastic pollution from shattered foam floats, and dependence on trash fish feeds. However, research on large yellow croaker aquaculture is limited. Through field surveys, we conducted an in-depth investigation of the status quo and practices of large yellow croaker farming in China, focusing on the major production provinces of Fujian and Zhejiang. Our research examined aquaculture methods, farming infrastructure, feed usage, and fish yields. We found cage farming in nearshore and estuarine areas dominates large yellow croaker production, while hatcheries and nurseries often use onshore indoor tanks. In recent years, driven by regulations, farms have gradually adopted modern plastic-framed cages with plastic floats, replacing wooden cages and foam floats. Some farms now use large High Density Polyethylene (HDPE) circular or square cages. The industry faces challenges including low product prices, high feed costs, personnel instability, frequent outbreaks of diseases and low fry survival rates. Trash fish feeds remain widespread, only switching to pellets during fishing moratorium or when trash fish prices are high, with an overall feed conversion ratio of 6.82. Our results indicate substantial room for improvement in Chinese large yellow croaker farming regarding personnel, fry survival, and dependence on trash fish. These findings provide valuable insights to enhance the sustainability of large yellow croaker farming in China.

**Keywords:** Cage Aquaculture; China; Field Survey; Large Yellow Croaker; Mariculture; Trash fish

## **Livelihood Vulnerability and its Influencing Factors of Retired Fishermen: A Case Study of Hunan and Hubei Province Based on the LVI-IPCC Framework**

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**Abstract:** Following the fishing-ban policy in the Yangtze River, retired fishermen have encountered substantial vulnerability in their livelihoods. This study utilizes the Livelihood Vulnerability Index (LVI) framework proposed by the Intergovernmental Panel on Climate Change (IPCC) to establish an assessment index system for evaluating the livelihood vulnerability of retired fishermen within the Yangtze River Basin. The research assesses the vulnerability of these fishermen's livelihoods, categorizes retired fishermen into different vulnerability levels through the Jenks natural break classification method, and employs a linear regression model alongside Shapley value decomposition to analyze the factors influencing their livelihood vulnerability. The findings of this study reveal the following key insights: (1) Overall, retired fishermen within the study area experience a notable level of livelihood vulnerability. Those in Hubei exhibit slightly higher vulnerability levels due to the influence of the job market and inherent limitations in their livelihood capital. (2) Retired fishermen, categorized into high, medium, and low livelihood vulnerability levels, exhibit distinctive traits in the unidimensional livelihood vulnerability index. The 'High level' group demonstrates 'high exposure, low adaptation, high sensitivity,' the 'Medium level' group shows 'medium exposure, medium adaptation, medium sensitivity,' while the 'Low level' group displays 'low exposure, high adaptation, low sensitivity.' (3) Several factors significantly influence the livelihood vulnerability of retired fishermen, including gender, age, education level of the household head, household annual income, and livelihood diversity. The factor of characteristics of householder contribute most to livelihood vulnerability. In light of these findings, it is imperative to implement targeted measures to alleviate the livelihood vulnerability of retired fishermen within the Yangtze River Basin. Such measures should encompass the enhancement of risk prevention mechanisms, the implementation of practical employment support activities, and the strengthening of livelihood capital. These steps are essential to reduce exposure and sensitivity while bolstering adaptive capacity, ultimately mitigating the livelihood vulnerability faced by retired fishermen in the Yangtze River Basin.

**Keywords:** Fishing-ban compensation policy of Yangtze River; LVI-IPCC framework; Livelihood Vulnerability; Jenks Natural Break Classification; Shapley value decomposition method

## **Research on the Purchase Intention of Wild Freshwater Aquatic Products under the Background of the Yangtze River Fishing Ban Policy**

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**Abstract:** The ten-year fishing ban policy is an important measure to cope with the rapid decline of aquatic biological resources in the Yangtze River Basin. Do consumers in the wild freshwater aquatic market trust or be skeptical? Are you willing to actively respond to the fishing ban policy to protect the fishery resources and ecological environment in the Yangtze River Basin, and reduce or even stop the purchase of wild freshwater aquatic products? Based on the survey data of eight provinces and cities in the Yangtze River Basin, this paper constructs a theoretical framework based on SOR theory, attribution theory and planned behavior theory, and discusses the influencing factors and formation mechanisms of consumers' purchase intention of wild freshwater aquatic products from the process of "external stimulus-internal psychological processing of the body-behavioral response". The results showed that the Yangtze River fishing ban policy, attitude, subjective norms, and perceptual behavior control all had a direct and significant impact on the purchase intention. Attitude plays a partial role as a mediator between subjective norms and purchase intentions; Consumers will have a trusting attitude towards the Yangtze River fishing ban, but the policy has a positive effect on consumers' willingness to buy, indicating that consumers will not actively respond to the policy to reduce purchases, but increase their purchases. The research conclusion will help the government propose targeted measures for market-related consumers to promote the protection of the ecological environment and fishery resources of the Yangtze River.

**Keywords:** Yangtze River fishing ban policy; Purchase intention; Structural equation model; SOR theory; Planning behavior theory