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Usage of advanced technology helps to get better results

Microorganisms have existed on the earth for more than 3.8 billion years and exhibit the greatest genetic and metabolic diversity. They are an essential component of the biosphere and serve an important role in the maintenance and sustainability of ecosystems. It is believed that they compose about 50% of the living biomass.

Dear Readers,

The October 2022 issue of *Aqua International* is in your hands. In the news section you may find news about ...

We published a special feature on the developments of an MOU between BKMN Aqua and ICAR-CIFA, Bhubaneshwar for the development and the production of Broodstock and Post Larvae of CIFA GI Scampi to expand the availability of the Genetically Improved Scampi seeds to the farmers in different locations of India as it can be cultured in freshwater as well as slightly brackish water (<7 ppt), which has the huge demand in domestic market as well as international market. BKMN Aqua promoted by Bijumon M.S., Kotaiah K and Maruthi Srinivasa Rao K are producing over 3200 Million seeds of SPF Vannamai, SPF Monodon and CIFA GI Scampi with 9 hatcheries in different parts of the country. They are also planning to start Aquaculture Academy to enhance the qualified professionals by giving free coaching and free boarding to the eligible candidates.

Dr Mahapatra, retired Principal Scientist, Kolkata Centre of ICAR-Central Institute of Fisheries Education addressing an event recently stated that fishery in India progressed from hunting to hobby; to farming to industry. Scientists in late 1950s thought about quality fish seed production, tank improvement programme begun with bottom silt cleaning, oxygen augmentation for increasing stocking density (SD) and fish production, pond bottom treatment (faecal matter management), bottom silt cleaning during culture (Fish Toilet). Steadily fisheries and Aq progressed as an industry - aeration with bottom cleaning, use of probiotics, RAS, aquaponics to absorb nitrite, Biofloc fish culture (nutrient recycling with probiotic and advantageous over conventional pond culture) and IMTA. He explained features of Aqua technologies for Entrepreneurship Development Programme. He also discussed principles of IMTA; *L. vannamei* monoculture with Better Management Practices in high SD; mud crab *Scylla olivacea* farming and fattening, cage culture/ box farming for producing soft-shelled crab; explained feed recycling pathway in Biofloc fish culture; principles of RAS, aquaponics; possible combinations of integrated farming with fish, its adoption percentage became higher with successful technology dissemination; fish farming in paddy plots.

E Hino Fernando, an expert on fisheries extension said that "Farm environment is better suited for GIF tilapia as there are chances of them mating with the in the event of females around. Culturing the males in a farm pond will help them direct their energy- -otherwise meant for mating- -towards increasing physical growth," said which deals with advising on farming practices for increased fish production and income. From the time they are procured from hatcheries and introduced into ponds, the fish achieve maturity in roughly about four months. They can be sold for meat in the fifth month onwards. A fisheries department official pointed to State government schemes such as the multipurpose farm pond scheme where farmers can get up to Rs 15,000 to set up farm ponds to rear such fish**In December 2014, Prime Minister Narendra Modi** called for a "blue revolution" and took several measures to harness the potential of fisheries. Some key measures include: The creation of a separate ministry of fisheries, animal husbandry and dairying; the formation of the department of fisheries with an independent administrative structure; bringing about policy reforms initiatives; and the creation of a fisheries and aquaculture infrastructure development fund in the financial year 2018-19 under the Atmanirbhar Bharat (self-reliant) package, aiming to double the income of small and artisanal fish farmers.

Contd on next page

NRS GROUP

Aqua International

Our Mission

Aqua International will strive to be the reliable source of information to aquaculture industry in India.

AI will give its opinion and suggest the industry what is needed in the interest of the stakeholders of the industry.

AI will strive to be The Forum to the Stakeholders of the industry for development and self-regulation.

AI will recognize the efforts and contribution of individuals, institutions and organizations for the development of aquaculture industry in the country through annual Awards presentation.

AI will strive to maintain quality and standards at all times.

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It aims to transform the sector holistically, focusing on production and productivity growth, increased domestic consumption and export earnings and reduced post-harvest losses. To enhance fish production and reduce post-harvest losses, the uptake of modern aquaculture, capture fishing and post-harvest management practices are essential.

Dhaval Contractor, Partner in Vaishnavi Aquatech, explained how the company's establishment of India's first state- of-the-art broodstock multiplication centre (BMC) for specific pathogen-free (SPF) monodon in 2021 would act as a catalyst for the rapid growth of the monodon sector. According to Contractor, who also produces feed and has 400 ha of shrimp ponds, monodon are becoming more appealing to farmers in several regions of India – in particular Gujarat – due to a range of factors adversely affecting L.vannamei production since 2017. These include disease issues (especially EHP), declining survival rates and increased competition from Ecuador's booming vannamei sector, leading to large-scale losses. "India is all set to become the [world's] leading producer of P. monodon. Bring back smile on the farmers' face; make aquaculture more sustainable in India, providing P. monodon shrimp to the world market, which has better texture and taste.

District Collector K Senthil Raj inaugurated the sea ranching near "Tharaipaar" close to Tsunami Nagar, Thoothukudi in Tamil Nadu. Appreciating the efforts of ICAR-CMFRI to support the sea-ranching of Indian pearl oysters for stock enhancement, he said that ICAR-CMFRI should continue the initiative until the pearl oyster stock grows to support pearl production. Pearl aquaculture and its associated activities could enhance the livelihood of coastal fishers and provide avenues for social engagement and well-being, especially for fisherwomen. In addition to the main spat release, two cages with different sizes of settled spat were deployed for regular monitoring of sea-ranched pearl oyster spat for its growth at this location. Local fishers of Thoothukudi district, notably women fishers from Sippikulam village, were trained by ICAR-CMFRI Scientists for the entrepreneurship development in pearl culture technique through funded research programmes.

Dr Shriparna Saxena, an aquaculture expert working in coordination with the forest department for two decades for the conservation of Mahseer, told PTI that a survey conducted in 1964 had showed that there used to be 25 Mahseer out of every 100 fish in the Narmada river. But due to the construction of many dams across the Narmada river and its tributaries and human intervention, the number of Mahseer in the Narmada has now decreased to less than one per cent, she claimed. "Fishermen living on the Narmada river banks say if lucky, they are able to spot a Mahseer once in six months," she informed. Under the Mukhyamantri Matsya Vikas Yojana, a programme to increase the Mahseer fish in the state is going to start from next month.

In the Articles section – *Pigment-Based Chemotaxonomy: A Modern Technique to Assess Phytoplankton Assemblages in the Aquatic Ecosystem* *authored by* Vivekanand Bharti, J. Jayasankar, T. V. Sathianandan of CMFRI, Ernakulam, Kerala, Shashi Bhushan, CIFE, Versova, Mumbai discussed that Phytoplankton considered as the primary producer, forms the basis of aquatic food webs, which all consumers of the ecosystem depend on either directly or indirectly for sustaining their lives. Consequently, the nutrition, growth, reproduction and survival of all aquatic organisms are influenced by phytoplankton assemblage. In addition to supporting the food web, phytoplankton monitoring offers essential information about eutrophication, biodiversity, harmful taxa and invasive species. Therefore, the knowledge of taxonomic composition and biomass of planktonic communities are key parameters to comprehend the energy flow pathways in aquatic food webs, and also to assess the water quality of the ecosystem. Many approaches have been evolved towards the taxonomy of phytoplankton over the years including morphological classification, anatomical classification and chemical classification.

Another article titled ***Mechanisms of Antimicrobial Resistance in Bacteria,*** *authored by* Petchimuthu M, Rujan J and Jaculine Pereira J of Dr MGR Fisheries College and Research Institute, TNJFU, Nagapattinam, said that Microorganisms have existed on the earth for more than 3.8 billion years and exhibit the greatest genetic and metabolic diversity. They are an essential component of the biosphere and serve an important role in the maintenance and sustainability of ecosystems. It is believed that they compose about 50% of the living biomass. In order to survive, they have evolved mechanisms that enable them to respond to selective pressure exerted by various environments and competitive challenges. The disease-causing microorganisms have particularly been vulnerable to man's selfishness for survival who has sought to deprive them of their habitat using antimicrobial agents. These microorganisms have responded by developing resistance mechanisms to fight off this offensive. Currently antimicrobial resistance among bacteria, viruses, parasites and other disease-causing organisms is a serious threat to infectious disease management globally.

Article titled ***Application Of Monoclonal Antibodies In Aquaculture,*** *authored by* Anurag Semwal, Ujjwala Upreti and Avdhesh Kumar, 123College of Fisheries Science, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, informed that Monoclonal antibodies (MAbs) are crucial reagents used in biomedical studies and developments, in analysis of diseases and their treatment like infections and malignant growth. MAbs are produced by clones or cell lines got from animals that have been inoculated / immunized with the substance. The cell lines are developed by intertwining B cells from the vaccinated animal with myeloma cells. For the production of ideal MAbs, the cells must be cultured in either of two ways: by in vitro tissue culture or by injection into the peritoneal cavity of a suitably prepared mouse (in vivo). Further proceeding of the mice ascitic fluid and of the tissue-culture supernatant might be needed to get MAbs with the prescribed purity and concentration.

Results in Shrimp, Fish and Crab farming can be achieved as per specifications when the pond management guidelines are followed. Farmers and Integrators have to give sufficient time and attention to farm management and check the developments there to ensure results. When you invest your hard earned money into it, a little more care and attention can prevent losses and help in profitable farming all the time.

Readers are invited to send their views and comments on the news, special feature and articles published in the magazine which would be published under "Readers Column". Time to time, we shall try to update you on various aspects of Aquaculture sector. Keep reading the magazine Aqua International regularly and update yourself. Wish you all

M.A.Nazeer
Editor & Publisher
Aqua International

India is on its way to be a global leader in fisheries

The Government of India has also launched its flagship scheme, Pradhan Mantri Matsya Sampada Yojana, with the highest ever investment of ₹20,050 crore in the fisheries sector

Fisheries play a crucial role in development. Considered a sunrise sector, it envisages bringing in great potential in an equitable, responsible, and inclusive manner. The sector employs approximately 28 million fish farmers and fishers and almost twice the number along the value chain.

In December 2014, Prime Minister (PM) Narendra Modi called for a "blue revolution" and took several measures to harness the potential of fisheries. Some key measures include: The creation of a separate ministry of fisheries, animal husbandry and dairying; the formation of the department of fisheries with an independent administrative structure; bringing about policy reforms initiatives; and the creation of a fisheries and aquaculture infrastructure development fund in FY 2018-19 worth ₹7,522.48 crore. So far, proposals worth ₹4,923.94 crore have been recommended to states and Union Territories (UTs), including 20 fishing harbours and 16 fish landing centres and 25 proposals from private beneficiaries worth ₹120.23 crore.

The Government of India (GoI) has also launched its flagship scheme, Pradhan Mantri Matsya Sampada Yojana (PMMSY), with the highest ever investment of ₹20,050 crore in the fisheries sector. PMMSY

Across the country, the scheme has got overwhelming responses from all states and UTs, and in the last two years, the department has sanctioned projects worth ₹ 8,562.72 crore for sectoral development.

was launched by the PM, on September 10, 2020, under the Atmanirbhar Bharat (self-reliant) package, aiming to double the incomes of small and artisanal fish farmers. It aims to transform the sector holistically, focusing on production and productivity growth, increased domestic consumption and export earnings, and reduced post-harvest losses. To enhance fish production and reduce post-harvest losses, the uptake of modern aquaculture, capture fishing, and post-harvest management practices are essential. For this, PMMSY lays special focus on skill and capacity-building.

Across the country, the scheme has got overwhelming responses from all states and UTs, and in the last two years, the department has sanctioned projects worth ₹8,562.72 crore for sectoral development. It is inspiring to share that fish production has increased from 141.64 lakh tonnes during 2019-20 to 162.53 lakh tonnes, as of date. On the other hand, India's fisheries exports stood at an all-time high of ₹57,586.48 crore. The Indian export market is dominated by shrimps, particularly L vannamei. To achieve the target of exports worth ₹1 lakh crore under PMMSY, the department has been focusing on diversifying the export basket by increasing the production and quality of tilapia, trout, pangasius and other species. The activities and projects sanctioned to date have generated employment for around 350,000 people directly, and over 970,000 across the value chain. The central assistance of ₹3,000 per beneficiary per year has provided livelihood and nutritional support to a total of 677,462 marginalised fish farmers and their families during the fishing ban/lean period.

To augment and replenish fish production, promote sustainable fisheries practices, and support bio-conversation, PMMSY has introduced a sea and river ranching programme. PMMSY aims to emphasise interventions where fishing vessel insurance, promoting sustainable aquaculture, extension support services, technology infusion, integrated aqua park building, and fisheries cooperatives are some of the components. PMMSY gives special emphasis on employment generation for women, Scheduled Castes, and Scheduled Tribes by providing alternate livelihood opportunities such as seaweed cultivation, ornamental fisheries. PMMSY provides 60% subsidies to women beneficiaries, including benefits to women entrepreneurs. Projects worth ₹1534.05 crore have been sanctioned for women, supporting 37,576 women beneficiaries.

Encouraging private sector participation, PMMSY has earmarked a separate fund of ₹100 crore under the entrepreneur models and urges young entrepreneurs to offer solutions through technology interventions. To facilitate access to institutional credit and meet working capital requirements, the GoI has extended kisan credit card (KCC) facilities to fish farmers from FY 2018-19. KCC national campaigns are being organised with the finance ministry and state departments. The national fisheries development board (NFDB), the nodal agency for PMMSY, has been organising fish festivals, culinary seminars, and exposure visits. The department released a book named Fish & Seafood – a collection of 75 gourmet recipes on August 10. Along with these interventions, the GoI has been making efforts to develop Indian fisheries towards becoming a global leader in the sustainable fisheries and aquaculture sector.

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Probiotics for water treatment

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3. DECREASE AMMONIA CONTENT

Prevent the accumulation of toxic substances such as NH_3, NO_2, etc.

4. IMPROVE WATER COLOR

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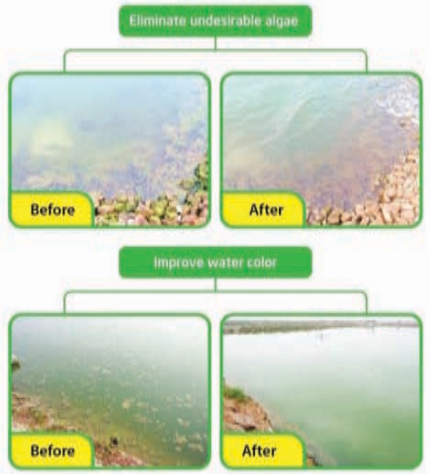
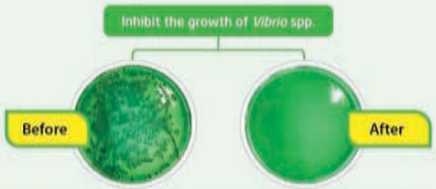


5. ESTABLISH BALANCED POND BACTERIA SYSTEM

Compete nutrition with vibrio and inhibit them to grow. Provide nutrition for probiotics in the pond, to establish a well-balanced farming system.



6. INCREASE AQUACULTURE PRODUCTION

Good quality of water prevents fish/prawn infections, making high profit of production

* COMPOSITION:

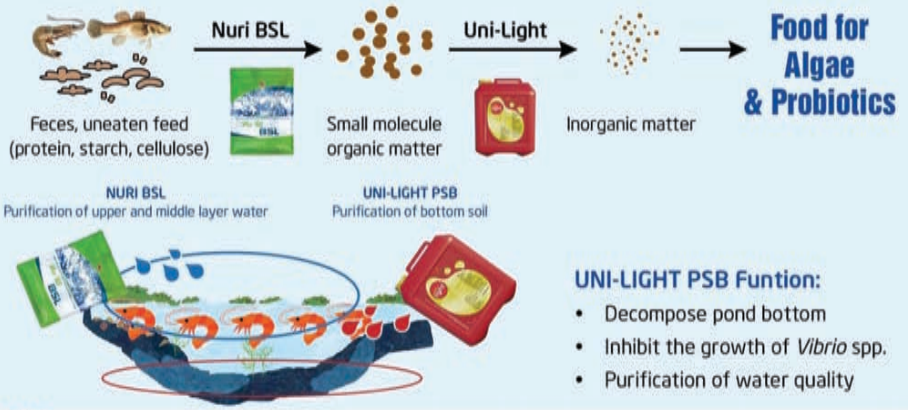
Bacillus spp. > 1x 10^{11} cfu/kg	15%
(Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus licheniformis)	
Carrier (rice bran, corn gluten)	75%
Moisture	10%

* STORAGE:

Keep at dry, well-ventilated condition. Avoid direct sunlight exposure and use as soon as possible once opened for best quality.

* DIRECTION OF USE:

No cultivation is needed. Apply Nuri BSL with water-soluble bag near to the working water wheel or pour into the pond evenly. Recommend apply Uni-Light PSB together with Nuri-BSL on sunny day to achieve a clear pond more efficiently.



UNI-LIGHT PSB Funtion:

- Decompose pond bottom
- Inhibit the growth of *Vibrio* spp.
- Purification of water quality

BSL Dosage:

/10,000 m³ Quantity	10 - 30 pl/m² tiger prawn or < 80 pl/m² Vannamei	For >30 pl/m² tiger prawn or > 80 pl/m² Vannamei	For > 150 pl/m² Vannamei
7 days before stocking	800 g - 1,000 g	1,200 - 1,500 g	1,200 - 1,500 g
Day of stocking	300 g - 500 g	800 g - 1,000 g	800 g - 1,000 g
Every 7 - 10 days after stocking	300 g - 500 g	800 g - 1,000 g	3 - 5 days / use 1,000g - 2,000g

****Dosages can be adjusted according to the water conditions and practices.*

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500,000 tonne monodon boom Predicted for India

India's tiger shrimp (Penaeus monodon) sector will grow to over 500,000 tonnes within the next five years, according to one the country's leading producers of the species.

Speaking at this week's Global Shrimp Forum, Dhaval Contractor, partner in Vaishnavi Aquatech, explained how the company's establishment of India's first state-of-the-art broodstock multiplication centre (BMC) for specific pathogen-free (SPF) monodon in 2021 would act as a catalyst for the rapid growth of the monodon sector.

According to Contractor, who also produces feed and has 400 ha of shrimp ponds, monodon are becoming more appealing to farmers in several regions of India – in particular Gujarat – due to a range of factors adversely affecting L.vannamei production since 2017. These include disease issues (especially EHP), declining survival rates and increased competition from Ecuador's booming vannamei sector, leading to large-scale losses.

While monodon production was in decline for many years – largely due to the use of poor quality wild seed, which grows slowly and is disease-prone – Contractor noted that the Moana monodon broodstock from the company's BMC were producing PLs that were performing much better and thus were gaining popularity among farmers.

Dhaval Contractor, partner in Vaishnavi Aquatech, speaking at the inaugural Global Shrimp Forum

He explained that in 2021 the company sold 300 million monodon post-larvae (PLs) to farmers in Gujarat and Andhra Pradesh and the demand was growing so quickly that they have now established six hatcheries and already sold more than 1 billion PLs in 2022.

India harvested a modest 34,000 tonnes of monodon in 2021-2022, but Contractor said that 70,000 tonnes is expected to be harvested in the 2022-2023 financial year and that this figure would be closer to half a million tonnes within five years.

Contractor outlined the marked improvements in the Moana monodon – which are now on their 19th generation – growth rates have increased to nearly 4 g per week, up from 2 g per week in 2008, and some farmers in Gujarat have been able to produce two crops a year, compared to one per year in the past.

Following years of decline India harvested a modest 34,000 tonnes of monodon in 2021-2022, but Contractor said that 70,000 tonnes is expected to be harvested in the 2022-2023 financial year and that this figure – at a conservative estimate – would be closer to half a million tonnes within five years.

Factors that add to the appeal of monodon, according to Contractor, are that they are a native species and therefore more resistant to pathogens such as EHP than vannamei; that they require less technology and infrastructure to produce; and that they can be grown to over 50 g and therefore fetch premium prices, especially on the Japanese and EU markets.

Aa a result of these factors Contractor predicts that 80-90 percent of farmers in Gujarat will grow monodon next year, while he is now supplying farmers in Orissa, West Bengal and Andhra Pradesh too.

"India is all set to become the [world's] leading producer of P. monodon. Bring back smile on the farmers' face; make aquaculture more sustainable in India, providing P. monodon shrimp to the world market, which has better texture and taste," he concluded.

India's CMFRI hosts release of hatchery-produced pearl oyster spat

The Central Marine Fisheries Research Institute, part of India's Department of Agriculture (ICAR-CMFRI) has released 500,000 farmed pearl oyster spat in the Gulf of Mannar.

The pearl oyster spat was released in the Gulf of Mannar on 15 September

Tuticorin Regional Station of the ICAR-CMFRI produced 5 lakhs of hatchery-produced pearl oyster (Pinctada fucata) spat and released the 5 mm seeds in selected areas of the Gulf of Mannar on 15 September 2022. This stock replenishment measure was initiated by the Shellfish Fisheries Division, ICAR-CMFRI aimed at restoring the depleted population of pearl oysters in the region

District Collector K Senthil Raj inaugurated the sea ranching near "Tharaipaar" close to Tsunami Nagar, Thoothukudi in Tamil Nadu. Appreciating the efforts of ICAR-CMFRI to support the sea-ranching of Indian pearl oysters for stock enhancement, he said that ICAR-CMFRI should continue the initiative until the pearl oyster stock grows to support pearl production. Pearl aquaculture and its associated activities could enhance the livelihood of coastal fishers and provide avenues for social engagement and well-being, especially for fisherwomen. In addition to the main spat release, two cages with different sizes of settled spat were deployed for regular monitoring of sea-ranched pearl oyster spat for its growth at this location.

Deploying the oyster baskets

Tuticorin is popularly known as "Pearl City" as it served as a capital of pearl production and trade centre for centuries until 1961 when the pearl fishery was completely banned by the Department of Fisheries to protect the dwindling pearl oyster stocks. Due to the huge demand for marine pearls, Tuticorin Regional Station of ICAR-CMFRI initiated research work on cultured pearl production and perfected the technology in 1973.

ICAR-CMFRI's oyster hatchery

Local fishers of Thoothukudi district, notably women fishers from Sippikulam village, were trained by ICAR-CMFRI Scientists for the entrepreneurship development in pearl culture technique through funded research programmes. The hatchery produced spat can be raised as an adult and used for pearl culture. ICAR-CMFRI has done commendable work on sea ranching of pearl oyster spat in the paars earlier. However, regular fishing activities in the paar areas kept the population under control without attaining its healthy stock status.

At present, ICAR-CMFRI is planning to rejuvenate the pearl oyster stocks in the pearl oyster paars of Gulf of Mannar through its sea ranching programme. Close monitoring will be carried out to assess its survival and sustainability through dedicated research activities. During the programme, the fishers from the Tsunami colony, who are descendants of pearl fishers and presently engaged in chank fishery, shared their experience of their ancestors and expressed their interest in carrying out pearl culture activity with the support of the Tuticorin Regional Station of ICAR-CMFRI.

Dr P S Asha, principal scientist and scientist-in-charge at the Tuticorin Regional Station of CMFRI, Scientist Smt M Kavitha and Scientist Dr C Kalidas also spoke on the occasion. Mr P Vijayaraghavan, assistant director of fisheries, Mrs Vyla, assistant director of fisheries, Mr Sivasubramanian, RDO, Mr Selvakumar, Tehsildar and other officials from the collectorate, scientists and technical staff of the Station and fishers from Tsunami Nagar were present at the function.

Madhya Pradesh: Mahseer fish Facing Existential Threat; State to Start Conservation Campaign from Next Month

But due to the construction of many dams across the Narmada river and its tributaries and human intervention, the number of Mahseer in the Narmada has now decreased to less than one per cent, she claimed.

The existence of Mahseer fish, popularly known as the tiger of freshwater, is under threat following increasing hindrances in the Narmada river's natural flow after the construction of various dams on it, a conservation expert said recently.

Taking cognisance of it, the Madhya Pradesh government has announced that it is going to start a campaign from next month to save the endangered Mahseer fish species. The Narmada river originates from Amarkantak and falls into the Gulf of Khambhat.

Dr Shriparna Saxena, an aquaculture expert working in coordination with the forest department for two decades for the conservation of Mahseer, told PTI that a survey conducted in 1964 had showed there used to be 25 Mahseer out of every 100 fish in the Narmada river.

But due to the construction of many dams across the Narmada river and its tributaries and human intervention, the number of Mahseer in the Narmada has now decreased to less than one per cent, she claimed. "Fishermen living on the Narmada river banks say if lucky, they are able to spot a Mahseer once in six months," she informed.

"We had found a five-feet four-inch long Mahseer weighing 17 kg in the river at Khalghat in Dhar district in 2017. We have not seen such a big fish till date," the expert said. Officials said major dams built on the Narmada in Madhya Pradesh include the projects of Bargi, Indira Sagar, Omkareshwar and Maheshwar, while the Sardar Sarovar dam has been built on this river in Gujarat.

Some other dams have also been built on the tributaries of Narmada, they said. Asked about the Mahseer fish facing existential crisis following the construction of reservoirs, MP Fishermen Welfare and Fisheries Development Department's principal secretary Kalpana Shrivastava said "dams are also necessary like Mahaseer."

Under the Mukhyamantri Matsya Vikas Yojana, a programme to increase the Mahseer fish in the state is going to start from next month, she said. Shrivastava expressed hope that the programme will lead to a big rise in the number of Mahseer in the next two years. State Fisheries Federation's Managing Director Purushottam Dhiman said that Mahseer fish seeds would be planted in Denwa, Tawa and other tributaries of the Narmada under the campaign.

"It is very important to have Mahseer in the Narmada from the point of view of biodiversity. Since it is a freshwater fish, its presence in the river is itself proof that its water is pure," he said. Dhiman said as part of steps to conserve Mahseer in the state, fishermen have been told that if this fish gets caught in their nets, then they should release it alive in the water.

Explained: Why Odisha Government has cancelled Shrimp farming in Bhitarkanika National Park

Over 95% of cultured shrimp produced in India is exported

The Fisheries department in Odisha has cancelled a lease agreement for shrimp farming. The agreement was signed between the state government and a seafood export company in which over 400 acres of land was supposed to be used for shrimp farming.

What is the matter?

The land granted for shrimp farming is at the seaside Banapada village which is situated within the eco-sensitive zone of Bhitarkanika National Park.

According to The New Indian Express, a petition was filed by RTI activist Ranjan Kumar Das in 2016 in which he alleged that the state government illegally leased out around 400 acres of land to a seafood export company for farming. The area is located within the eco-sensitive zone of Bhitarkanika and the move also violates the Coastal Regulation Zone (CRZ) and other laws.

During the hearing, the Odisha Lokayukta was informed by the director of fisheries that the Fisheries department cancelled the lease agreement after the "rationalisation of the boundary" of Bhitarkanika by the Forest department in 2022. With this order from the Fisheries director, the Lokayukta closed the case.

How is shrimp farming affect the region?

The residents of the area told the New Indian Express that due to the release of untreated effluent from shrimp farms, the fertile agricultural land in the village has been damaged. The same area was known as "the rice bowl" of the region.

With the introduction of farming in the region, it turned into a small island surrounded by shrimp farms. It is also threatening the nearby rich mangrove forests. Locals claimed that the land has lost its fertility and turned into barren land.

Bhitarkanika Wildlife Sanctuary

Famous for crocodile conservation and thick mangrove forests, the Bhitarkanika National Park and Wildlife Sanctuary is located in Kendrapara district in Odisha. After Chilika Lake, it is the second Ramsar site in Odisha and is also the second largest mangrove ecosystem in India.

Gahirmatha Beach and Marine Sanctuary are to the east of the national park and is inundated by the rivers Brahmani, Baitarani, Dhamra, and Pathsala.

Shrimp industry in India

In 2020, PM Modi launched the Pradhan Mantri Matsya Sampada Yojana (PMMSY) for five years (2020-25), which also aims at increasing the production of shrimp and achieve a goal of producing 1.4 million tonnes by 2024.

According to the Hindu Businessline, the government also reduced the import duty on certain input products for shrimp aquaculture from 30 % to 10 % to benefit farmers. Reports states, "the market attained a volume of 0.71 million tonnes in 2022 and it will grow at a CAGR of 9.5 per cent and reach 1.23 million tonnes by 2026."

According to The Hindu, the US and China are the biggest importers of Indian Shrimp. India exported 8,93,644 tonnes of shrimp to the US in 2021, marking a 20 per cent growth.

India's major shrimp-producing states are Andhra Pradesh, West Bengal, Odisha, Gujarat and Tamil Nadu. Significantly, over 95% of the cultured shrimp produced is exported.

Gassen Plus

Bon Ammonia and obnoxious Gasses

Shrimp / Fish performs all their body functions and growth in water. Good quality water and proper D.O. levels determines the success or failure. Good quality water, optimum D.O. level is of prime importance for health and growth of Shrimp / Fish.

Irregular water exchange, excess and leftout feed, dead algae, fecal matter, increases the organic load at the pond bottom. Accumulation of such waste absorbs available oxygen, creating anaerobic condition which leads to pollution of pond bottom. Polluted pond bottom and unhealthy environmental conditions triggers the release of toxic gasses like Ammonia, H_2S, Methane, etc, The toxicity of Ammonia, Hydrogen Sulphide, Methane attributed mainly due to unionized form. As the concentration in water increases, ammonia excretion by aquatic organism diminishes and the level of ammonia in blood and in other tissues increases. Ammonia increases oxygen consumption by tissues, damage gills and reduces the ability of blood to transport oxygen, and increases the disease susceptibility. To eliminate / overcome the above problems 'GASSEN PLUS' Yucca Schidigera, it contains Steroidal"Saponin" which help to reduce ammonia and other noxious gasses such as H_2S, Methane, etc., Microbial enzyme "Urease' Production inhibited by Saponin which leads to an increases D.O. and reduction of BODand COD levels.

Bacterial strains such as Bacillus Subtilis, Nitrobactor, Nitrasomonas, rapidly converts ammonia into Nitrates, Nitrites and finally non-toxic Nitrogen. Hydrogen Sulphide converts into Sulphates, Sulphites and finally non-toxic Sulphur, Methane into Non-toxic carbon. This conversion reduces the obnoxious gasses in the pond bottom. Reduction of this gasses improve the D.O. level in the water and bottom.

COMPOSITION:
YUCCA SCHIDIGERA
ALOEVERA
BACILLUS SUBTILIS
BACILLUS POLYMIXA
BACILLUS LICHENIFORMIS
NITRASOMONAS
NITROBACTOR
STABILIZERS

DOSAGE : 1 Kg per Acre or consult your Aqua Technician For Specific Usage & Dosage

PRESENTATION: 500 gms &1 kg

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Prof S. Banerjee Memorial Lecture on Entrepreneurship Development through Aquaculture

The first Prof Samir Banerjee Memorial Lecture was organized by Fish Endocrinology Research Unit, Department of Zoology (DZ), University of Calcutta (CU), Kolkata in association with International Academy of Science & Research on August 30 2022 at Wallace Hall of DZ. It was delivered audio-visually by Dr Bijay Kali Mahapatra, Retd. Principal Scientist, Kolkata Centre of ICAR-Central Institute of Fisheries Education on 'Entrepreneurship Development through Aquaculture'. Late Prof Banerjee (died on 1.1.2017) was Hiralal Chaudhuri Professor of Zoology at CU and established Aquaculture Research Unit in DZ.

Dr Mahapatra stated that fishery in India progressed from hunting to hobby to farming to industry. Scientists in late 1950s thought about quality fish seed production, tank improvement programme begun with bottom silt cleaning, oxygen augmentation for increasing stocking density (SD) and fish production, pond bottom treatment (faecal matter management), bottom silt cleaning during culture ('Fish Toilet'). Steadily fisheries and Aq progressed as industry - aeration with bottom cleaning, use of probiotics, RAS, aquaponics to absorb nitrite, Biofloc fish culture (nutrient recycling with probiotic and advantageous over conventional pond culture) and IMTA. He explained features of Aq technologies for Entrepreneurship Development Programme (EDP), *viz.*, composite fish culture {with and without giant freshwater prawn (GFP)}; innovative ideas (application of palm leaves, bamboo branches, *Ipomoea* sp as prawn hideouts in ponds after moulting); features and production levels of monoculture of male GFP, Amur carp, GIFT; polyculture of major carps with desi Magur or Singhi without common carp (CC); monoculture of desi Koi, pabda *Ompak bimaculatus*, murrels, Jayanti Rohu; integrated culture of major carps with Small Indigenous Fishes (Mourola, *Chela* sp, *Puntius* sp); freshwater (FW) pearl culture; seabass farming in FW and brackishwater ponds; standardization of milkfish breeding technique and culture; polyculture of mullets; culture potential of *Mystus gulio*; brackishwater polyculture (BP) with milkfish, pearl spot and *Litopenaeus vannamei*; periphyton-based BP and substrates used for periphyton growth.

He continued discussing principles of IMTA; *L. vannamei* monoculture with Better Management Practices in high SD; mud crab *Scylla olivacea* farming and fattening, cage culture/ box farming for producing soft-shelled crab; explained feed recycling pathway in Biofloc fish culture; principles of RAS, aquaponics; possible combinations of integrated farming with fish, its adoption percentage became higher with successful technology dissemination; fish farming in paddy plots. He elaborately discussed about EDP and livelihood opportunities in ornamental (aquarium) fishery sector. A *Channa barca* costs about Rs 200000/- in international market. Opportunities include export of wild-caught indigenous FW ornamental (Orn) fishes, aquarium plant propagation, aquarium accessories making, fish trade, breeding & seed production and rearing of exotic FW Orn fishes upto adult stage. Wild-collected weed fishes (barbs, etc) are Indian Orn fishes, regarded as 'swimming dollar'. Similarly *Badis badis* is high-priced in international market; Indian glass fish *Chanda ranga*, sucker-mouth crocodile fish in cold torrential rivers, *Mastacembelus* sp, *Macrognathus* sp, *Rita rita* as white catfish in aquarium, devil catfish *Chaca chaca*, familiar killifish *Aplocheilus panchax* are highly preferred and high-priced indigenous Orn fishes of high demand. Orn fishes may be reared in nylon net hapa enclosures in old ponds in villages and sub-urban areas; new intervention is goldfish culture in rectangular cages or hapa in major carp ponds.

Different varieties of goldfish, molly, guppy (high-priced with big caudal fin), swordtail, fighter fish produced applying genetics & breeding principles. Inbreeding (brother-sister mating) is unwanted for important cultivable FW foodfishes but beauty observed when applied for Orn fishes. Expression of new genes is possible leading to uncommon body shape/ feature. Dr Mahapatra highlighted on his angelfish breeding success, first time in India. He closely observed its breeding behaviour, identified brood male and female, found fertilized eggs deposited on filter tube in aquarium tank. He spoke about effective low-cost technologies, *viz.*, rearing fighter fish individually in empty hospital saline bottles; guppy breeding in discarded empty earthen sweet curd container ('maateer bhaar' in Bengali; 1-2kg capacity) where gravid guppy gave birth to young ones; old silk dupatta ('orna') of females modified as zooplankton (*Daphnia* sp) collection device from ponds; adult guppy collected from wastewater canals; produced seeds raised to adulthood and sold.

As entrepreneurial avenues, he has hands-on experience on breeding of gourami, cichlids, zebrafish; standardized *Colisa lalia* breeding and seed production in rectangular cement cisterns using *Vallisneria* plant and thermocol pieces. Indigenous Orn fish *C. ranga* bought from us by scientists/technologists at Singapore and Thailand at low price, do genetic manipulation leading

to very attractive body feature (vibrant colour) and we buy improved fish from them at very high price (Rs 100-150/-/piece). Albino magur holds good potential as Orn fish. He also bred goldfish and Koi carp using mops in cement cisterns, Corydora catfish, blood parrot fish and livebearers; did breeding of high-priced *Andinoacara* sp, Discus using discarded ice-cream cones. Live food collection, culture and trade also included in EDP. He furthermore discussed breeding technique of indigenous brackishwater Orn fishes (orange chromidae, *Scatophagus argus*, etc); flow-through tubifex culture system; infusorians culture using banana peel-offs; mosquito larvae culture as Orn fish food; their artificial feed preparation method (successive steps from selecting feed ingredients till pellet drying); aquarium plant propagation and trade; features of aquascaping. He developed mini fish breeding chamber at home (6ft x 2ft x 1ft big glass tank) for few indigenous high-priced foodfishes, where conditions of big hatchery are simulated.

Towards the end, Dr Mahapatra discussed about novel breeding & seed production technologies of indigenous Magur, Pabda catfish, *M. gulio*, *M. vittatus* in eco-hatchery, of CC in winter in cloth hapa in ponds, of Amur carp, *Piaractus brachypomus* by stripping, as EDP. Controlled breeding and mass-scale seed production of Singhi is now possible by simulating natural environment and creating water current, without sacrificing males. He inspired MSc students and researchers in DZ as participants, also News communicator Subrato Ghosh, on EDP with much of his own pioneering works and ideas and influential remark: 'Work silently, your work will speak'. It was illuminating and enriching presentation, good exposure for participants on available options for entrepreneurs on various avenues of inland Aq and fisheries sector in West Bengal.

Genetically improved tilapia aquaculture ideal 'GIFT' for Nagapattinam fish farmers: ICAR-KVK

Places where the Cauvery or rainwater recharges the ponds in the district are best suitable for GIFT culture as it is a freshwater fish, he added.

Nagapattinam: At a time when inland aquaculture is finding its feet in the district that is otherwise largely dependent on marine fishing, ICAR-Krishi Vigyan Kendra in Sikkal is adding impetus to the former by promoting genetically improved farmed tilapia (GIFT) among fish farmers. Experts say the fish variety when bred can grow fast and yield farmers profit in a shorter period.

"Genetically improved farmed tilapia are those type of tilapia fish which are bred selectively and improved in its characteristics such as faster growth, disease resistance, and tolerance to a wide range of water quality parameters, "

ICAR-KVK experts and fish farmers displaying genetically improved tilapia at a farm pond in Nagapattinam district

said Dr A Gopalakannan, ICAR-KVK programme coordinator.

Places where the Cauvery or rainwater recharges the ponds in the district are best suitable for GIFT culture as it is a freshwater fish, he added. As the fish is cultured for meat, experts say that GIFT chosen for breeding is all-male. It is appropriate to culture only males and keep females as broodstock, they added, advising farmers to procure only male fishlings from hatcheries. They also advise culturing them in a closed farm environment like a farm pond or a fish pond than in natural environment like a village pond.

"Farm environment is better suited for GIF tilapia as there are chances of them mating with the in the event of females around. Culturing the males in a farm pond will help them direct their energy--otherwise meant for mating--towards increasing physical growth," said E Hino Fernando, an expert on fisheries extension, which deals with advising on farming practices for increased fish production and income.

From the time they are procured from hatcheries and introduced into ponds, the fish achieve maturity in roughly about four months. They can be sold for meat in the fifth month onwards. A fisheries department official pointed to State government schemes such as the multipurpose farm pond scheme where farmers can get up to Rs 15,000 to set up farm ponds to rear such fish. A Pazhaniappan, a fish farmer from Naluvedapathy in Thalaignayiru block said, "I can harvest GIF tilapia fish twice a year and fetch a profitable price during sale. I get double the profit by selling during the annual fishing ban."

BKMN Aqua targets to make GI Scampi seed available to most of farmers in Inland regions to produce huge tonnage with limited production cost

BKMN Aqua had an MOU with ICAR-CIFA for the development and the production of Broodstock and Post Larvae of CIFA GI Scampi to expand the availability of the Genetically Improved Scampi seeds to the farmers in different locations of India. BKMN is producing 3200 to 3950 million seed of SPF Vannamei, SPF Monodon and CIFA GI Scampi with 9 hatcheries in the country.

Aqua International Editor, M.A. Nazeer had an exclusive interview with the partners of BKMN Aqua Mr Bijumon M.S, Mr Kotaiah. K and Mr Maruthi Srinivasa Rao K. Excerpts:

Successful together: **Kotaiah. K, Bijumon M.S and Maruthi Srinivasa Rao. K, partners of BKMN Aqua**

Aqua International: Where is your company's Head Quarters ?

BKMN Aqua: BKMN Aqua Headquarter is located at 1190/1,2,6, Srikakulam Village, Ghantasala Mandal, Krishna District, Andhra Pradesh.

AI: Who are its Partners?

BKMN: Mr Bijumon M.S, Mr Kotaiah. K and Mr Maruthi Srinivasa Rao. All the 3 are in the field of aquaculture since 1995.

AI: Where do you have your shrimp seed production units (hatcheries) ?

BKMN: BKMN Aqua has nine hatcheries, RS No 1190/1,2,6, Srikakulam Village, Ghantasala Mandal, Krishna District in Andhra Pradesh.

BKMN Aqua, Ramudupalen Village, Challapalli Mandal, Krishna Dist.

BKMN Aqua, near Ex CM Residency, Amaravathi Karkatta Road, Undavalli Tadepalli Mandal, Guntur Dist.

Bkmn Aqua, Vajjireddy Palem Village, Kothapatnam, Prakasam Dist.

Bkmn Aqua, Ramudupalem Village, Mudivarthipalem Post, Indukurpet Mandal, Nellore Dist.

Bkmn Aqua, Gangapatnam Village, Indukurpet Mandal, Nellore Dist.

Bkmn Aqua, Nr Tupilipalem Beach, Tupilipalem Village, Vakadu Mandal, Nellore Dist.

BKMN Aqua, Nr Vagarru, Tupilipalem Village, Vakadu Mandal, Nellore Dist.

Bkmn Aqua, Anna Nagar, Koneru Kuppam, Chengalpattu Dist, Tamil Nadu.

AI: What kind of tie up you have with CIFA for your project ?

BKMN Aqua: BKMN Aqua had an MOU with CIFA for the development and the production of Broodstock and Post Larvae of CIFA GI Scampi to expand the availability of Genetically Improved Scampi seeds to all the farmers in the different locations of India as it can be cultured in freshwater as well as slightly brackish water (<7 ppt) which has the huge demand in domestic market as well as international market.

AI: What are the best practices / aspects you have to produce quality shrimp seed and to provide services to farmers?

BKMN: Consistent supply of quality seeds to aqua farmers with the motto of "Your Success is Our Goal". Continuously adapting the advanced techniques and the machinery which leads to the best water quality and the best quality of the seeds. As per the impressions and the feedback of the farmers, we improve our production techniques.

We import different types of broodstock which are genetically improved.

We maintain different lines of seeds and supply the line of seeds which are apt for the region by continuously getting the feedback and field survey that which line is suitable for the particular region.

AI: What are your future plans and targets in Aquaculture industry ?

BKMN Aqua: We don't want to depend on the same species. As we have a lot of hatcheries, we want to diversify the production of different species. We started with scampi initially and later on we diverted our production to Vannamei and then Monodon. We are planning to adapt to the new technologies by working along with the research institutes. We are working with the research institutes and the government wings like CIFA and CIBA to produce the GI Scampi, Seabass, mud Crab and Milkfish in the future. Meanwhile, we are planning to educate the farmers to diversify their culture practices for producing different species. Trout Fish is also the future food. We are doing research on the culture practices of the Trout fish now for getting the higher yields in the lesser crop period.

Especially, we are aiming on the GI Scampi as their growth rate is more. India has a lot of fresh water lands. Making use of fresh water ponds in the inland regions to produce the fresh water

Bijumon M.S

Kotaiah. K

Maruthi Srinivasa Rao. K

Abouth the Promoters

Mr Bijumon M.S:
He belongs to the Aleppy, Kerala, which have a lot of brackish water region. During his childhood, he used to spend a lot of time with his parents while catching the aquatic animals. From that time he was interested about the aquatic animals and their habitat. With the huge interest on this subject, he studied about the aquatics and gradually it has become his profession. During his early days he worked with the fish and the shrimp hatcheries, and farms and later on got an idea to start his own shrimp hatchery. Later they expanded their hatcheries at multiple locations to an extent to produce the multiple species.

Mr Kotaiah. K:
He is well experienced in the farming and in the field of hatcheries while India is doing the culture of Monodon and Scampi. During his days in the time of 2000s he met with the remaining partners and formed BKMN Aqua.

Mr Maruthi Srinivasa Rao. K:
Earlier, Maruthi Srinivasa Rao was in the textile industry. As there are a lot of ups and down in the textile industry, during that time some of his friends who are already in the aquaculture industry suggested him to start shrimp farms as there is huge profits in aquaculture. Later, he started his own aquaculture farms along with the trading of the different types of materials, equipments, feeds and the chemicals which are related to the aquaculture. During that time he met his partners Mr Bijumon and Mr Kotaiah and together formed a company BKMN Aqua which deals with the production of multiple species.

species like GI Scampi. Our agenda and target for the next 5 years is to make GI Scampi seeds available for the most of the farmers in the inland regions for producing huge tonnage with limited production cost.

To Start Aquaculture Academy

We are planning to start the Aquaculture Academy to enhance the qualified professionals by giving the free coaching and free boarding to the eligible candidates.

Establishing the Research lab to accommodate the qualified research scholars and to develop the genetically improves species of the aquatic animals for getting the best yields to the farmers in the less span of time.

Sitting from Left to Right: ***Salman Raju, Head-Technician, Maruthi Srinivasa Rao. K, Kotaiah. K, Bijumon M.S, promoters and Sujith Kumar P.V, Chief Administrative Officer and Core Team members of BKMN Aqua.***

Scampi is back as 'CIFA GI Scampi'

Giant freshwater prawn, *Macrobrachium rosenbergii* (Scampi) is an indigenous species of India inhabiting rivers, canals, estuaries and coastal waters. It is one of the most important cultivable species of freshwater systems due to its high price, large size, faster growth, good taste and high export demand. It is cultured in freshwater as well as slightly brackish water (<7 ppt) and can be cultured alone (monoculture) or with compatible species (polyculture) like rohu, catla and Chinese carps like silver and grass carp. ICAR-CIFA has developed a genetically improved and fast growing scampi through genetic selection. The selection programme was started in the year 2007 and in 2019, 11th generation of fast growing scampi was produced with an average selection response of 7% per generation or a cumulative response of 70%. The new fast growing variety was developed from three different stocks of scampi collected from Gujarat, Kerala and Odisha. Every year around 50 to 60 families are produced by selecting the largest males and females from the previous generation families. This procedure is repeated every year as the generation interval of scampi is one year. Prawns from each family are tagged with Visible Implant Alpha (VIA) numeric tags to identify them and to have pedigree information for genetic selection. This newly developed fastgrowing scampi are also found to be performing better in farmers fields in Odisha and Andhra Pradesh. A package of practices for scientific culture of this improved scampi was also developed.

Post Larvae

BKMN Aqua had an MOU with CIFA for the development and the production of Broodstock and Post Larvae of CIFA GI Scampi to expand the availability of the Genetically Improved Scampi seeds to all the farmers in the different locations of the India as it can be cultured in freshwater as well as slightly brackish water (<7 ppt) which has the huge demand in domestic market as well as international market.

Why to culture Genetically Improved Scampi?

Productivity of genetically improved scampi is 70-80% higher than other un-improved stock. Therefore, the adaption of genetically improved scampi can increase production, enhance profitability and improve income of aqua farmers.India has most of the inland freshwater sources where the culture of GI Scampi can get the higher yields to boost the sea food industry of the country. The material requirement of the scampi is huge in the international market as well as domestic market as it grows to biggest size which can be sold at premium prices.

Shrimp Post Larvae

Genetically improved seed availability

Genetically improved Scampi seeds are available at BKMN Aqua, Nellore. BKMN Aqua is now boosting their production capacities of Genetically Improved Scampi for wider availability of the GI Scampi seeds to most of the aqua farmers in the different parts of the country.

Slow Sand Filters

Package of practices for culture

Basically the package of practices for culture of improved scampi/prawn is similar to that of un-improved scampi. It can be cultured in existing fish ponds or in new ponds. While constructing a new pond the following points are to be noted.

Post Larvae Packing

New pond construction

Culture site where water temperature remains above 200C for 6- 7 months are suitable. Select site that have a reliable source of good quality freshwater / brackish water [<7ppt] with pH above 7.0 and alkalinity above 100 ppm. Pond bottom soil should be clayey loam or sandy loam. Pond should be preferably rectangular in shape and size of 0.2-1.0 ha is easy to manage. Provision of inlet and outlet and water control structures should be there for ease of operation. Pond bottom should have suitable slope towards the outlet. Depth of water should be maintained at minimum of 1.0-1.5m (3-5 feet). Smaller pondswhich can easily be drained to harvest prawns are always preferable.

Pond preparation

Ponds should be drainable, dried and bottom should be exposed to sunlight for atleast a week, those that are non-drainable should be applied commercial bleaching powder (30% chlorine) @ 350 kg/ha-m of water at least three weeks before seed stocking. Once the pond is dried, agriculture lime is applied on the bottom soil @ 200 kg/ha if thesoil pH is 7; if the soil pH is less than 7 then the rate of lime needs to be increased. Water is properly filtered and filled in the pond upto a level of 1.0 m in nursery and 1.5 m grow out pond. Phased manuring with a mixture of groundnut oil cake at 250 kg, cow dung / vermicompost 70 kg and single super phosphate 17 kg/ha have shownto be effective in production of desired plankton. A thick paste of half of the above quantities are prepared by addition of sufficient water and applied as basal dose 2-3 days prior to stocking. The same dose isrepeated later preferably at five days' intervals depending on the plankton levels of the ponds. As prawns grow by moulting (shedding of outer shell) are very soft and can be easily eaten by other prawns. Earthen pipes, small tree branches, tyres, pvc pipes etc. are provided in the pond as hide out to save them from predators during moulting.Pond is covered with nylon net or thread to save prawns from predatory birds. Fencing around the ponds should be arranged well tomake sure that other animals likes snakes and crabs etc., should not enter into the pond.

Nursery pond management

After two weeks of pond preparation healthy genetically improved post larvae (PL) procured from BKMN Aqua should be stocked after proper acclimatization. The nursery rearing can be done in small earthen ponds, cemented tanks or in nylon hapas. The size of the nursery pond can range from 0.02 to 0.1 ha. The recommended stocking density of post larvae (seed) ranges from 50-100 per m2. In case of cemented tanks water should be aerated and water exchangeshould be done at least once in a week. In case of hapa rearing, hapa should be changed fortnightly to avoid clogging. Provision of floating weeds inside a PVC frame covering 10% of the pond water area is recommended to provide shade and shelter to PL. Commercially available scampi/shrimp feeds (starter feed in crumbled form) is recommended for good growth and survival. It should be fed with 100% biomass per day for first two weeks and reduced to 20% of the biomass towards the end. If the farmer doesn't have access to commercial feed, then powdered groundnut oil cake and rice bran can also be used. Nursery period may range from 45 to 60 days during which the PL grows to juvenile upto 2 - 5g size with a survival rate of 80-85% with proper feeding and water quality management.

Farm Reared CIFA GI Scampi 168 Grams

Grow out pond management

Juveniles of 2 - 5 g harvested from nursery ponds are to be seggregated gender wise. The seggregated males and females are to be stocked seperately in well prepared larger grow out ponds of 0.1 to 1.0 ha. If bleaching powder is used as disinfectant during pond preparation, it must be ensured that toxicity is reduced and oxygen balance is established in the pond prior to seed stocking. For monoculture practice male prawns can be stocked @ 50,000- 60,000

/ Ha juveniles and female prawns can be stocked @ 70,000- 80,000 / Ha while for polyculture 10,000-15,000 / Ha prawn juveniles along with 6,000-7,000 fingerlings of catla and rohu are stocked. Polyculture of carp and prawn has the advantage that both prawn and carp utilize different food niches effectively. Polyculture of carp and prawn is recommended for higher productivity and income. The recommended fish species composition

BKMN Aqua, LRT Section

Farm Grown CIFA GI Scampi - 200 Grams

is catla and rohu at 1:1 or 1:2 depending on pond productivity. The post-stocking fertilization measures includes fortnightly application of dolomite and fermented groundnut oil cakealong with jaggery in case the water is clear and transparency is morethan 40 cm. Regular application of fermented groundnut oil cake and the rice bran will increase the zoo plankton in the pond. Highest plankton in the ponds leads to lesser costs of commercial feeds. The organic manure and inorganic fertilizers are applied in alternative weeks to maintain the natural productivity status.

Water quality management

Water transparency (visibility) and colour of the pond is an important indicator of the health of pond ecosystem. In unproductive pond the visibility can upto the bottom which will lead to growth of bottom algae that adversely affect the growth and survival of the prawns. Ideally the visibility should be maintained in the range of 30-40 cm to avoid water quality deterioration. Prawns are very sensitive to low dissolved oxygen in water. When the oxygen level in the pond is critically low (<3 ppm) the prawns come to the surface along the periphery which indicate the need for taking immediate remedial actions such as water exchange or operation of aerators to avoid mortality of stock. It

CIFA GI Scampi - 150 Grms

is necessary to replace at least 30% of water at regular intervals, if the water quality deteriorates during the later part of the culture. Provision of pond water aeration (by paddle wheel aerator or pump operation) is recommended especially during the final 2-3 months when the biomass in the pond is higher. Aeration during morning hours and during the cloudy days helps better oxygenation of the pond water. During grow out culture period water quality need to be maintained at optimum levels (DO:>4 ppm, pH:7-8, alkalinity above 100 ppm and transparency: 30-40 cm) for higher survival and growth.

CIFA GI Scampi - Female with Eggs

Feed management

Feed management is an important aspect of aquaculture. Excess feeding leads to wastage of feed and deterioration of water quality, while lack of feed/under feeding leads to cannibalism in prawns or slow growth. Prawns are fed twice daily with commercial prawn or shrimp feed @ 5-6% of their biomass which is gradually reduced to 2%towards the end of the culture period in monoculture. However, only floating fish feed @ 5-6% of body weight of fish should be given two split doses initially and then gradually reduced to 2 % towards the end of the culture period in polyculture system. There is no need of separate prawn or shrimp feed to be fed in the polyculture system. Feed is broadcasted into the pond from dyke. Sinking feed can also be given in check trays placed 2-3 Mtr. away from the dyke for better feed management. In the absence of

CIFA GI Scampi Broodstock - Male (Bigger Claws), Female

commercial feed in polyculture, mixture of groundnut/mustard oil cake and rice bran at 1:1 by weight fortified with vitamins and minerals can also be given in two split doses daily and provided in dough form preferably in feed trays or gunny bags hung at uniform distance inside the pond. Feeding should be reduced during cloudy days and winter. Regular monthly sampling needs to be carried out to assess the growth and health of the stock as well as to revise the daily feeding ratio.

CIFA GI Scampi Broodstock - Male (Bigger Claws), Female

Health management

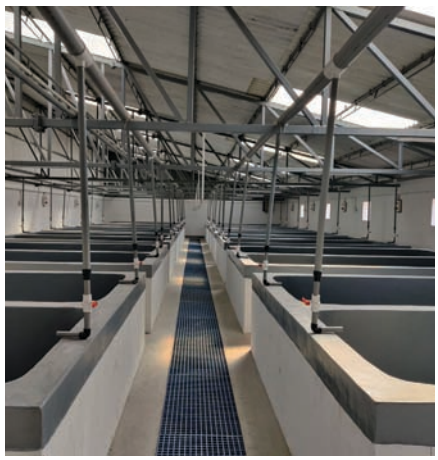
Poor rearing conditions like low water depth, excess stocking, over feeding, silting etc. are responsible for the disease problems in freshwater prawn culture. Loss of appendages, brown or black colouration/patches on shell, fouling on body are some of the symptoms seen in diseased prawn. If these symptoms noted, water should be exchanged (30-50%), reduce the stocking density, water quality should be tested and immediately consult the expert to avoid any loss of stock due to disease.

Farm Grown CIFA GI Scampi - 120 Grams

Harvesting

Fishes/Prawns attaining the marketable size can be harvested periodically to reduce the density of animals in the pond and hereby providing sufficient space for the growth of other stocked individuals. Partial harvesting to remove larger prawns (>40g) may start from 4th month of culture using seine net of suitable mesh size and this shouldcontinue every month till final harvest. This will allow faster growth ofsmaller ones with more space and food. The remaining prawns and carps can be finally harvested by netting followed by complete draining of the pond. In monoculture, at a stocking density of 5 nos./m2 the average final size after 6 months of culture would be about 80g if good quality pellet feed is provided to the prawns. Final survival rate of 70-80% is expected and the production may range from 2500 to 3000 kg/ha. However, in polyculture, prawn survival is expected to be more than 80 % with average size more than 60g with a production of 600-700 kg/ha of prawn and about 5.0 tonne/ha of fish. The carp and prawn polyculture has been proved to be highly profitable with low production cost.

CIFA GI Scampi - 65 to 70 Grams



Larval Rearing Tanks

Farmers and interested aquaprenuers can call at Toll Free number 18005726232 for sales of CIFA GI Scampi seeds, technical assistance about GI Scampi farming and other queries,

For Sales enquiries call at 9603769095, 8179751745

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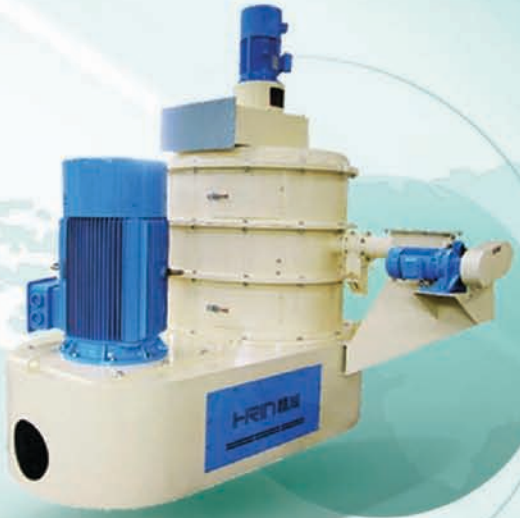

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Pigment-Based Chemotaxonomy: A Modern Technique to Assess Phytoplankton Assemblages in the Aquatic Ecosystem

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Highlight Points

- Morphological, anatomical and chemical characteristics are used to know the composition of phytoplankton
- Approaches such as light microscopy, scanning or transmission electron microscopy, spectrophotometry and chemical constituents may be applied in phytoplankton taxonomy
- Chemotaxonomy is an easy and convenient technique to accomplish the composition and biomass of phytoplankton
- Chemotaxonomy can assist the aqua-entrepreneur in monitoring and management of aquaculture and culture-based fisheries in large aquatic ecosystems such as ponds, lakes, wetlands and enclosures in open water bodies

Introduction

Phytoplankton, considered as the primary producer, forms the basis of aquatic food webs, which all consumers of the ecosystem depend on either directly or indirectly for sustaining their lives. Consequently, the nutrition, growth, reproduction and survival of all aquatic organisms are influenced by phytoplankton assemblage. In addition to supporting the food web, phytoplankton monitoring offers essential information about eutrophication, biodiversity, harmful taxa and invasive species. Therefore, the knowledge of taxonomic composition and biomass of planktonic communities are key parameters to comprehend the energy flow pathways in aquatic food webs, and also to assess the water quality of the ecosystem. Many approaches have been evolved towards the taxonomy of phytoplankton over the years including morphological classification, anatomical classification and chemical classification. The first two *i.e.* morphological classification and anatomical classification can be grouped under traditional classifications, whereas the third one is considered as a modern approach to classify the phytoplankton. In chemical classification, the chemical constituents of phytoplankton are used for its classification, hence it is known as chemical taxonomy or chemotaxonomy. The samples to be examined can be dried or crushed in chemotaxonomy classification, and therefore, the freshness or intact samples are not the mandatory requirements like morphological classification. Further, easy and convenient operational methodology in chemotaxonomy makes it a better method of classification in comparison to the traditional method.

Identification of macro-algae is relatively easy, but identification of microorganisms like nano and pico algae is quite a challenging task for phycologist. Also, with modernization of science, lack of interest in plant morphology resulted in a shortage of trained taxonomists, improper knowledge on laws of classification and terminology are major hurdles in the identification of phytoplankton. The development of chemotaxonomy has been proved one of the important tools to resolve this problem. The classification of plants with chemotaxonomic perspective started in the early twentieth century, where volatile oils and alkaloids in angiosperms were studied, but further development took place with the tune of paper chromatography in the mid-twentieth century, which made the comparison of samples quick and simple. But, rapid advancement gained momentum during 1970-1980 in techniques of phytochemistry, when capillary column (or high resolution) gas-liquid chromatography (GLC), HPLC, mass spectrometry and nuclear magnetic resonance paved the way for the development of this line of approach.

Qualitative and quantitative investigation of phytoplankton is often assisted by analysis of photosynthetic and photoprotective pigments, which are restricted to one or two taxa and can be used as marker pigments for those taxa (Table 1). All the living organisms produce secondary metabolites that are derived from primary metabolites. The chemical structure of the secondary metabolites and their biosynthetic pathways is often specific and restricted to taxonomically related organisms and hence useful in classification.

Table 1. Example of phytoplankton marker pigments used in chemotaxonomy

Pigment	Corresponding group
Chlorophyll-a	Total algal biomass (including cyanobacteria)
Chlorophyll-b	Chlorophytes, prasinophytes
19'-Butanoyloxyfucoxanthin	Chrysophyceae, haptophyta
Divinyl chlorophyll-a	*Prochlorococcus* sp.
Fucoxanthin	Diatoms, haptophyta, chrysophyceae
19'-Hexanoyloxyfucoxanthin	Haptophyta
Peridinin	Autotrophic dinoflagellates
Zeaxanthin	Cyanobacteria, *Prochlorococcus* sp.

Source: Rao *et al.*, 2018

Analytical techniques used in phytoplankton identification

There are several techniques for the identification of phytoplankton based on morphology, anatomy and pigments as shown in figure 1.

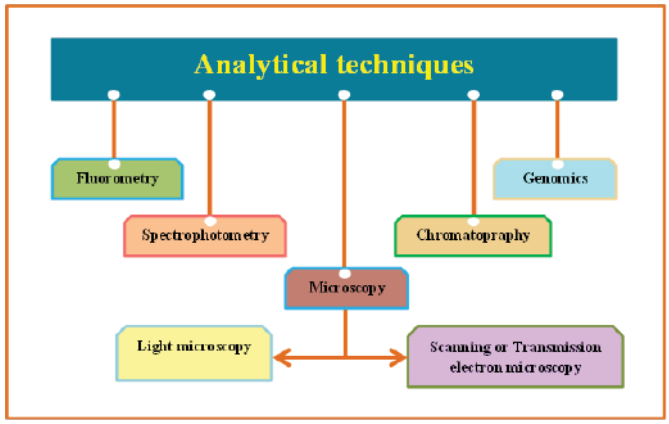

Fig. 1. Techniques for phytoplankton identification

1. Microscopy

Since the early stage of phytoplankton documentation, microscopy is the most used procedure to enumerate it. This traditional method is highly time-consuming, thus limiting the number of samples which can be analyzed. The limitation in analyzing the sample through microscopy, researchers required a long period to finish the project. Additionally, the use of fixatives to preserve samples introduces biases in the biovolume estimates due to cell shrinkage. In the consideration of the involvement of the microscope for in phytoplankton identification, there several types of the microscope are used ranging from a simple light microscope to the most sophisticated electron microscope. But, on the basis of the illuminating agent, the microscopy can be broadly classified into the following:

a. Light microscopy

Traditionally, a compound light microscope is engaged for the identification and estimation of cell abundance and biovolume. This approach of identification requires much time and highly skillful professionals. Despite strong efforts, results may vary notably among specialists. In addition to rising bias and error in assessing phytoplanktonic taxa, light microscopy does not offer to analyze the small-sized (<3 µm) algae *i.e.* pico phytoplankton. However, the contribution of autotrophic pico phytoplankton to total primary production may reach 50-90% in oligotrophic lakes and oceans, and 30-70% in mesotrophic or eutrophic lakes.

b. Scanning or transmission electron microscopy

Scanning or transmission electron microscopy uses electrons instead of photons to view phytoplankton in-depth, making it capable of capturing the variations between species of the same genus. The increased resolution of scanning or transmission electron microscopy allows identification of the pico phytoplankton, but the processes of sample preparation for electron microscopy are extremely time-consuming, especially in the case of the collection of sample for phytoplankton identification in large-scale surveys. Furthermore, the sample must be completely dry before placing it within the microscope. Biological samples are saturated in water, therefore as they are dehydrated, they begin to decompose and shrink up into unidentifiable masses. The chance of this problem increases in the case of working with marine life, as loss of seawater is often leaves

salt crystals in the outer membrane, which may hide the observation of some features. Some flagellates, which are soft-bodied, do not tend to preserve well during electron microscopy processing and are difficult to properly classify due to the destruction of key identifying morphological traits.

2. Spectrophotometry and fluorometry

Phytoplankton spectral absorption can be governed by the composition and concentration of pigments. Chlorophyll-a (chl-a) concentrations are widely used to estimate primary productivity by aquatic ecologists due to a proxy for phytoplankton biomass. The distribution of chl-a has been studied by spectrophotometry and fluorometry, these methods suffer from inaccuracies associated with spectral interferences from chl-b, carotenoids and from chl-a degradation products (e.g. chlorophyllides, phaeophytins and phaeophorbides), which may occur during senescence, grazing, sedimentation and re-suspension of phytoplankton. Moreover, accurate estimates of absorption by conventional spectrophotometers are difficult because of relatively dilute concentrations of particulate matter in natural waters sample, which is below the detection limits of laboratory spectrophotometers when measured in standard 1- or 10-cm cuvettes.

3. Genomics

In the recent period, the uses of molecular techniques (e.g., real-time qPCR, microarrays) are continuously increasing to study the evolutionary and biodiversity of planktonic organisms in the aquatic environment. However, the application of these techniques was started by a physiologist in 1970s decade. Although these methods offer foremost advances in taxonomic accuracy over standard microscope analyses, further development is needed before the complete replacement of conventional microscopy for typical diversity analysis in natural plankton assemblages. Moreover, a large number of molecular techniques are available as the application of all molecular tools is not straightforward for all kinds of research objectives. Furthermore, different techniques have their own particular strengths and weaknesses in methodological aspects like resolution, costs and technical expertise. The ability to reveal genetic markers for particular traits also differs between the various techniques. Also, species may differ by their genetic variation at particular loci. Hence, a technique that reveals polymorphisms in one species will not necessarily reveal the same level of polymorphism in another species.

4. Chromatography

The development of HPLC is considered the "yardstick" for measuring pigment concentrations in plant and algal samples. HPLC can determine the most chlorophylls and carotenoids compounds, including chl-a, degradation products such as chlorophyllides, phaeophytins and phaeophorbides, provided that relevant pigment standards would be available. The presence of characteristic pigments, mostly carotenoids, in algal phyla, is used for the identification of phytoplankton. Some pigments are characteristic of specific phytoplankton groups and can be used as diagnostic markers to classify phytoplankton assemblage. The application of phytoplankton's chemical constituent in its classification is termed as phytoplankton chemotaxonomy. This powerful technique for phytoplankton taxonomy was developed by Mackey *et al.* (1996), which can be used in both oligotrophic and more eutrophic coastal areas and estuaries. In oceanography, chemotaxonomic analysis with HPLC has been applied since the 1980's decade to assess marine phytoplankton pigment.

Total phytoplankton standing stock is estimated by knowing the chl-a concentration and algae classes are identified from the presence of diagnostic carotenoid pigments. Furthermore, the estimation of phytoplankton biomass of each taxon is achieved by analyzing the ratios of marker pigment to chl-a with the help of a matrix factorization program called CHEMTAX. A limitation of the pigment-based assessment method is that it is unable to provide high taxonomic resolution beyond the class level. Therefore, it has been suggested to use chemotaxonomy together with rapid microscopic screening to gain more specific information about the dominant species.

Steps involve in chemotaxonomy

According to Tamm *et al.* (2015), there are following steps for pigment based chemotaxonomy (Fig. 2):

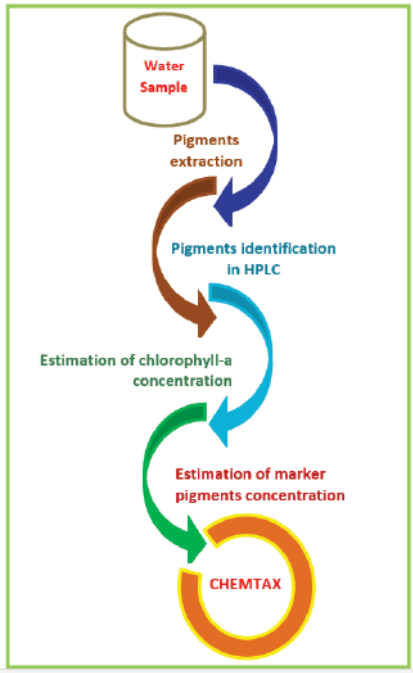

Fig. 2. Flowchart of chemotaxonomy procedure

I. Photosynthetic pigments are extracted from filters with acetone.

II. Sonicated for 5 minutes and kept at -20 °C in darkness for 24 hours.

III. Extracts are filtered through 0.45 µm syringe filters and stored in darkness at -20 °C for few hours or until HPLC analysis.

IV. Run HPLC to separate the phytoplankton pigments.

V. Absorbance spectra and retention times are recorded.

VI. Concentrations calculation of pigments comparing their retention times and peak areas with pure pigment standards.

VII. Chl-a concentration, as an indicator of the total phytoplankton biomass, are estimated.

VIII. Marker pigments concentration is observed.

IX. CHEMTAX is applied to know about microalgae assemblage.

CHEMTAX

CHEMTAX is a computer program that allocates chl-a, which is a proxy for phytoplankton biomass, into different algal groups on the basis of pigment markers. In this program, an iterative process is used to find the optimal pigment: chl-a ratios for generating the fraction of each pigment-determined group from total chl-a pool. Therefore, the estimation of phytoplankton composition is usually achieved by combining HPLC and a matrix factorization program called CHEMTAX. CHEMTAX is one of the most robust methods for analyzing pigment markers, which use factor analysis and a steepest descent algorithm to identify the best fit to the data based on initial estimates of the most appropriate pigment ratios for each phytoplankton class. This analysis depends on a data matrix of pigment concentrations and initial estimates of the most appropriate ratio of pigment: chl-a for the phytoplankton classes that might be expected in the samples. The software modifies each positive element of the pigment: chl-a ratio by a specific factor (usually 10-20%). CHEMTAX calculates the contribution from different phytoplankton groups to total Chl-a, based on the ratios between accessory pigments and Chl-a, which are loaded in the program together with pigment concentrations. However, the pigment concentration and the pigment/Chl-a ratios vary between species and groups and are influenced by light and nutrients. But, all CHEMTAX calculations first require normalization against total pigment. CHEMTAX uses a factorial analysis approach in which a matrix of pigment data is factorised in two matrices. The first expresses the marker pigments: Chl-a ratios for each of the different phytoplankton groups, and the second gives the abundance of each phytoplankton group in the sample from which the contribution of each group to the total Chl-a can be calculated.

Advantage of chemotaxonomy

I. Chemotaxonomy is faster and also more precise than microscopic analysis.

II. Much of the work in chemotaxonomy can be automated.

III. It can be used in a routine procedure.

IV. HPLC analysis of phytoplankton under the idea that pigment analysis, provides high consistency between users and laboratories with low variability and high reproducibility.

V. Another advantage of the pigment method is that all phytoplankton groups can be detected in one step because of the retention of all algal cells by filtration prior to HPLC analysis.

VI. HPLC analyses encompass all the phytoplankton cell sizes in the sample, while microscopy does not allow the identification of the smallest cells, *i.e.* the pico phytoplankton.

VII. Chemotaxonomy allows the identification of pigments that are specific biomarkers for some of the major taxonomic groups, like dinophytes, chlorophytes, diatoms, prasinophytes and cryptophytes. Hence, it provides us a good indication of phytoplankton community structure.

VIII. The Chl-a biomass of the individual phytoplankton groups can be calculated from pigment concentrations.

IX. Unlike morphological, optical, genetic or biochemical methods, the pigment-based method is suitable for regular monitoring as well as ecological studies.

Limitation

I. It requires previous knowledge of taxonomic composition and pigment ratios of the samples under analysis.

II. It does not provide high taxonomic resolution beyond the class level.

Conclusion

Chemotaxonomy is a better method of phytoplaplanton classification in comparison to the traditional methods. Besides automation of the maximum number of processes, the procedures involved in sample preparation are easy and convenient, wherein a relatively shorter time is required for routine analysis of phytoplankton dynamics by this modern approach. Chemotaxonomy can be used to monitor an oligotrophic aquatic ecosystem, where a high concentration of pico phytoplankton is found. Therefore, unlike morphological, optical and genetic methods, the pigment-based method is suitable for regular monitoring of the aquatic ecosystem. Chemotaxonomy can assist the aqua-entrepreneur in the management of aquaculture and culture-based fisheries in large aquatic ecosystems such as ponds, lakes, wetlands and enclosures in open water bodies.

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Mechanisms of Antimicrobial Resistance in Bacteria

Highlight Points

- Antimicrobial resistance develops when microorganisms change when they are exposed to antimicrobial drugs.
- The main cause of antimicrobial resistance is due to indiscriminate use of antibiotics. When we use antibiotics, some bacteria can survive and even multiply.
- The frequent use of antibiotics, the more chances bacteria have to become resistant to them. Intrinsic resistance, circumstantial resistance and acquired resistance are the types of resistance found in animals.
- The main mechanisms of resistance in bacteria cells are limiting uptake of a drug, modification of a drug target, inactivation of drug, active efflux of a drug and genetic transformation like mutation.
- Horizontal gene transfer, or the process of swapping genetic material between neighbouring contemporary bacteria, is a means by which resistance can be acquired.
- Antimicrobial resistance is one of the biggest threats to global health, food security today. It can affect anyone, of any age in any country. The understanding about antimicrobial resistance and its mechanisms helps to take the preventive measure against antibiotic resistance.

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1. Introduction

Microorganisms have existed on the earth for more than 3.8 billion years and exhibit the greatest genetic and metabolic diversity. They are an essential component of the biosphere and serve an important role in the maintenance and sustainability of ecosystems. It is believed that they compose about 50% of the living biomass. In order to survive, they have evolved mechanisms that enable them to respond to selective pressure exerted by various environments and competitive challenges. The disease-causing microorganisms have particularly been vulnerable to man's selfishness for survival who has sought to deprive them of their habitat using antimicrobial agents. These microorganisms have responded by developing resistance mechanisms to fight off this offensive. Currently antimicrobial resistance among bacteria, viruses, parasites, and other disease-causing organisms is a serious threat to infectious disease management globally.

2. Antimicrobial Resistance

The World Health Organization (WHO) defines antimicrobial resistance as a microorganism's resistance to an antimicrobial drug that was once able to treat an infection by that microorganism. Resistant microbes are more difficult to treat, requiring higher doses, or alternative medications which may prove more toxic. These approaches may also be more expensive. Microbes resistant to multiple antimicrobials are called multidrug resistant (MDR). Resistance is a property of the microbe, not a person or other organism infected by a microbe. Antibiotic resistance is a subset of antimicrobial resistance.

3. Principle of Antimicrobial Resistance in Bacteria

Several factors have been reported to be responsible to antibiotics resistance in bacteria. Some of the reasons includes: Reduced access to target due to slow porin channels; increased antibiotics expulsion due to multiple drug efflux pumps; inactivating enzymes due to β-lactamases, aminoglycoside-modifying enzymes; mutational resistance due to regulatory mutations that increases the expression of intrinsic genes and operons which is variable in certain circumstances (Nikkado *et al.*, 2003). The antimicrobial agents in widespread clinical use were developed to inhibit targets unique to prokaryotic cells such as bacterial cell wall, the bacterial ribosome and bacterial DNA gyrase. These antibiotics have reduced the mortality resulting from infectious diseases. Use and often abuse of antibiotics has encouraged the evolution of bacterial towards resistance, resulting often in therapeutic failure. Resistance reflects the ability of a microorganism to avoid the inhibitory and lethal activity of antimicrobial agents. (Fraimow and Abrutyn, 1995).

4. Types of Resistance

4.1. Intrinsic Resistance

Whereby microorganisms naturally do not possess target sites for the drugs and therefore the drug does not affect them or they naturally have low permeability to those agents because of the differences in the chemical nature of the drug and the microbial membrane structures especially for those that require entry into the microbial cell in order to affect their action. With intrinsic resistance the organism possesses properties that make it naturally resistant to certain insults, e.g. the more complex outer layer of gram-negative bacteria makes it much more difficult for certain antimicrobials to penetrate. It is considered to be a natural and inherited property with high predictability. Once the identity of the organism is known, the aspects of its anti-microbial resistance are also recognized.

4.2. Circumstantial Resistance

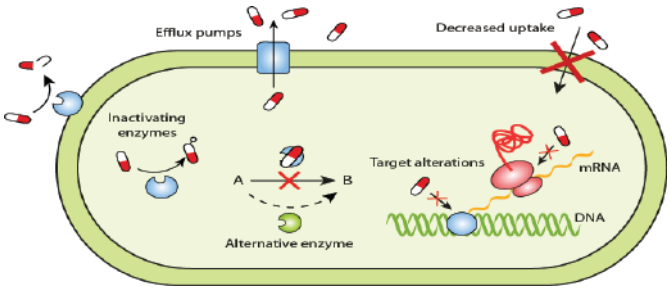
It is the difference between the *in vitro* and *in vivo* effects of an antimicrobial agent. Agents that appear to be active in the laboratory may be ineffective *in vivo* because of failure to reach the site of infection, such as the inability of first generation cephalosporin to cross the blood-brain barrier.

4.3. Acquired Resistance

Acquired resistance is when a naturally susceptible microorganism acquires ways of not being affected by the drug. Any insult, physical or chemical, has the potential to induce changes in the organism. Microbes are more ubiquitous however, and can actually acquire resistance from each other by sharing genetic material. They can pass genetic material from one to another in various ways; thus, microbes have been performing their own genetic modification for millions of years.

5. Mechanism of Antimicrobial Resistance

The major resistance mechanisms of microbes are decreased drug uptake, efflux pumps, enzymes that inactivate an antimicrobial chemical and target alterations by mutation. There also are biofilms.



5.1. Decreased uptake

As stated above the more complex outer layer of gram-negative bacteria make it much more difficult for certain antimicrobials to penetrate. Gram positive bacteria have a cell wall composed mostly of peptidoglycan, a very rigid substance. This is a prime target of β lactam antimicrobials such as penicillin and cephalosporin. The antimicrobial locks on to the β lactam structure in the cell wall, preventing expansion, and the cell ruptures as it grows. Gram negative bacteria have a much thinner cell wall itself and this is protected by a lipopolysaccharide molecule in the capsule, an outer membrane and what is known as the periplasmic space. In short it is a much more heavily armoured vehicle. Porins are openings in the cytoplasm membrane through which antimicrobial agents can gain entry a reduced number of such porins is one means of antimicrobial resistance.

5.2. Efflux pumps

Some bacteria like *Pseudomonas*, have a system called an efflux pump. As its name suggests this is a system whereby the bacterium has a pump to expel ingested chemicals. Although some of these drug efflux pumps transport specific substrates, many are transporters of multiple substrates. Antimicrobial efflux pumps are believed to contribute significantly to acquire bacterial resistance because of very broad variety of substrates they recognize, their expression in important pathogens, and their cooperation with other mechanisms of resistance, such as decreased uptake. Their presence also explains high-level intrinsic resistances found in specific organisms. The designs of specific, potent efflux pump inhibitors appear to be an important goal for the improved control of infectious diseases in the near future. For example, in ear therapy tris-EDTA has the potential to partially inactivate the efflux pump but this is only a topical specified action not generally available in most situations.

5.3. Enzyme inactivation

Some microorganisms have developed the ability to produce enzymes that are able to inactivate certain antimicrobials. The most notable example is penicillinase that can inactivate penicillin, but there are others. Clavulanic acid can bind penicillinase leaving the antimicrobial amoxicillin to do its work, and also there are the penicillinase resistant penicillin such as methicillin and cloxacillin, but they are still subject to target alterations making them ineffective over time.

5.4. Mutation

When an antimicrobial attack a specific target, whether it be cell wall peptides, ribosome or nuclear DNA, it locks on to specific receptors on the target. Bacterial mutation results in the alteration of these receptors so that the antimicrobial can no longer fit and the organism is thus resistant to the effects of the antimicrobial.

6. Mechanisms of Resistance Gene Transfer

Drug resistance may be acquired by passage of the trait vertically to daughter cells, but more commonly it is acquired by horizontal transfer of resistance by, Transduction, Transformation and Conjugation.

6.1. Transformation

Transformation refers to the ability of microorganisms to utilise snippets of free DNA from their surroundings. DNA from dead cells gets cut into fragments and exits the cell. The free-floating DNA can then be picked up by competent cells. Exogenous DNA is taken up into the recipient cell from its surroundings through the cell membrane. The exogenous DNA is incorporated into the host cell chromosome via recombination. Transformation results in the genetic alteration of the recipient cell.

6.2. Transduction

Transduction is acquisition of bacterial DNA from a phage that has incorporated DNA from a previous resistant host bacterium. e.g. strains of *S. aureus*. Bacteriophages can transmit genetic material from one organism to another.

6.3. Conjugation

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. It is a mechanism of horizontal gene transfer as are transformation and transduction although these two other mechanisms do not involve cell-to-cell contact.

6.3.1. Transposons

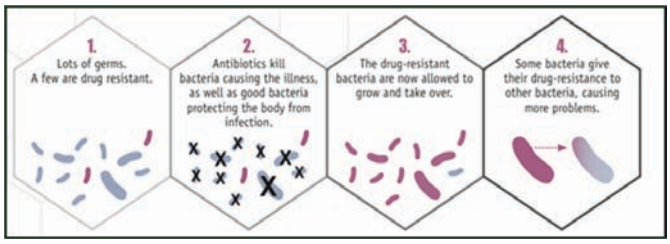
Transposons are sequences of DNA that can move around different positions within the genome of single cell. The donor plasmid containing the Transposons, co-integrate with acceptor plasmid. They can replicate during co integration. Both plasmids then separate and each contains the r-gene carrying the transposon. e.g. *Staphylococci, Enterococci*

6.3.2. Integrons

Integron is a large mobile DNA can spread Multidrug resistance. Each integron is packed with multiple gene cassettes, each consisting of a resistance gene attached to a small recognition site. These genes encode several bacterial functions including resistance and virulence, it cannot promote self-transfer.

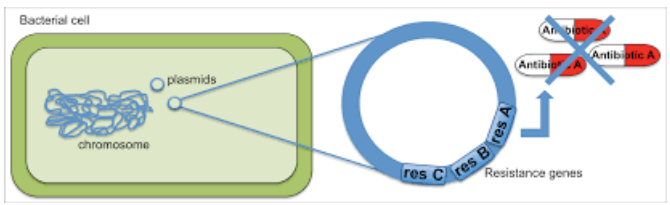
7. Selection for Resistance

Susceptible organisms are eliminated leaving resistant ones to multiply the pathogens. Organisms may be partially resistant and susceptible to higher levels of antimicrobials.



8. Co-Selection for Resistance

A crucial factor is the fact that integrons often carry the resistance genes for several anti-microbial at the same time. Thus, overuse of a less crucial antimicrobial, such as tetracycline may result not only in selection for resistance to tetracycline but also to other, possibly more critically important, antimicrobials. This is highly relevant as it means that, while overuse of antimicrobials deemed critically important should always be avoided.



9. Prevention of Antimicrobial Resistance

Resistance of bacteria against antimicrobial substance gives more strength for survival. Resistant microbes are more difficult to treat, requiring higher doses, or alternative medications which may prove more toxic. So, we have to reduce the resistance capacity of bacteria against antimicrobial substance. The following steps can be preventing the antimicrobial resistance of bacteria.

- No indiscriminate and inadequate or unduly prolonged use of antimicrobial agents.
- Prefer rapidly acting and selective (narrow spectrum) AMAs.
- Use combination of AMAs for prolonged therapy e.g. tuberculosis,
- Intensive treatment for notorious organisms.

Conclusion

Antimicrobial resistance is one of the biggest threats to global health and food security. It can affect anyone, of any age in any country. It occurs naturally, but misuse of antibiotics in human and animals is accelerating the process. So, proper understanding needs about how antimicrobial resistance develops the principles of horizontal gene transfer, selection for resistance and the interaction between humans and animals. This understanding helps to take the preventive measure against antibiotic resistance, So We can minimise resistance to antimicrobial therapy.

APPLICATION OF MONOCLONAL ANTIBODIES IN AQUACULTURE

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Introduction

Monoclonal antibodies (MAbs) are crucial reagents used in biomedical studies and developments, in analysis of diseases and their treatmentlike infections and malignant growth. MAbsare produced by clones or cell lines got from animals that have been inoculated/immunized with the substance. The cell lines are developed by intertwining B cells from the vaccinated animal with myeloma cells. For the production of ideal MAbs, the cells must be cultured in either of two ways: by in vitro tissue culture or by injection into the peritoneal cavity of a suitably prepared mouse (in vivo). Further proceeding of the mice ascitic fluid and of the tissue-culture supernatant might be needed to getMAbs with the prescribed purity and concentration.

In last two decades, Aquaculture have been set up as an industry in India. Due to intensive culture of species (fish and shellfish), chance of disease and economic loss have been continuously increasing in rearing and grow out ponds.Hence, to maintain the aquaculture industry, development of diagnostics and vaccines are very important for proper health management.Evolution of specific, sensitive and rapid diagnostic methods are very essential to determinedifferent stages of disease like per acute, acute to sub-acute and chronic infection. In addition, there is a need to develop basic and delicate strategiesfor diagnosing pathogens for epidemiological study. At present diagnosis is performed in numerous research centersusing the regular biochemical strategieswhich are repetitive, costlyand even not effectively sensitive to differentiate the large number of heterogeneous isolates. Development of effective vaccines for disease prevention is another area of interest in health management.Since an enormous numberof isolates of variousvirulence and stereotypes exists in a disease situation, there is a need to recognize general immunogenic immunogens shared by different isolates to develop a proper polyvalent vaccine.

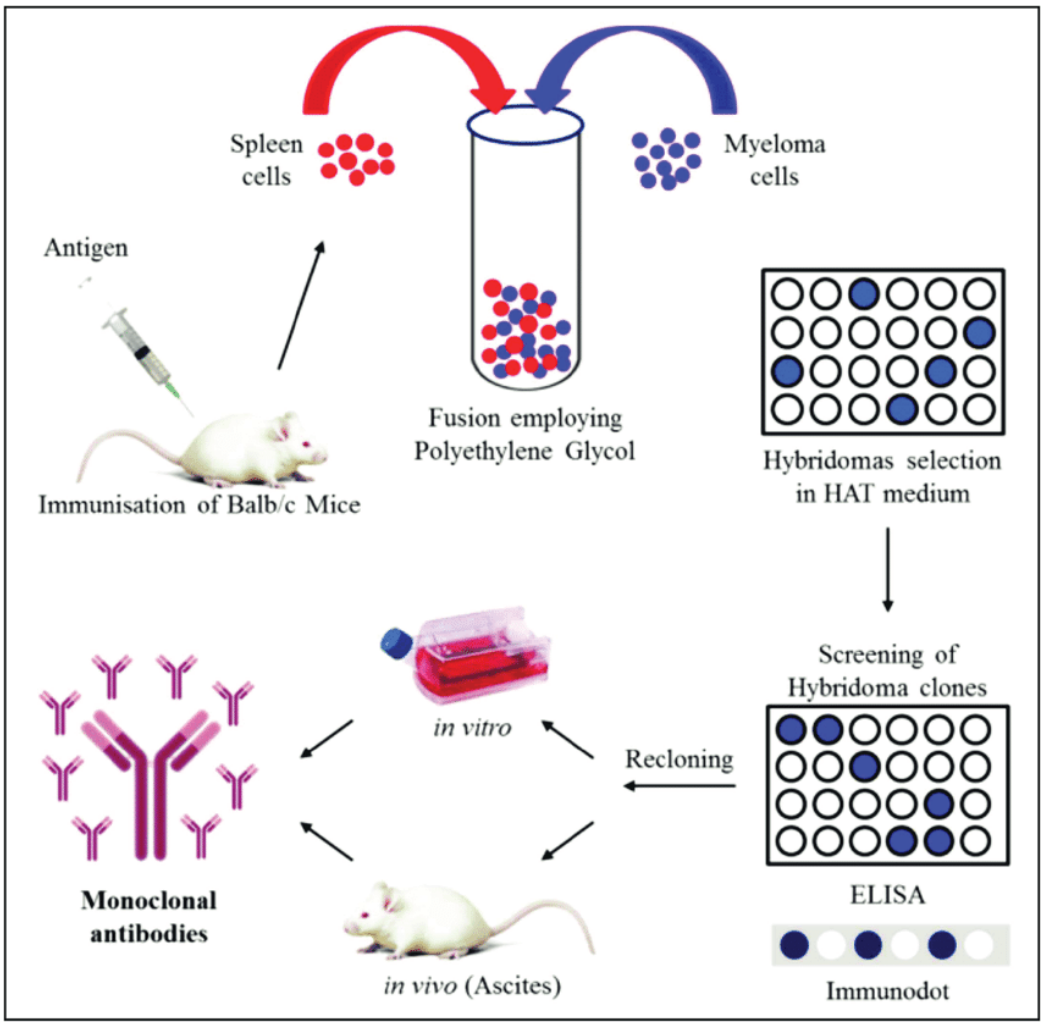
Highlight Points

- Monoclonal antibodies (MAbs) are being utilized in pathogen classification, disease diagnosis, epidemiological investigation and improvement of vaccines.
- Monoclonal antibodies will bind to the specific target molecule, without any undesirable side effects.
- MAbs based ELISA have been used for studies of *Vibrio anguillarum* strains and for investigation of clinical cases of furunculosis (*Aeromonas salmonicidae*) and Enteric Red mouth (*Yersinia ruckeri*) in fish farms.

Production of Monoclonal antibody

Monoclonal antibodies are generally made by combining myeloma cells with the spleen cells from a mice/rabbit that has been vaccinated with the desired immunogen. Polyethylene glycol is utilized to intertwineadjacent plasma membranes, but the effectiveness is low.Along these,a selective medium in which only combined cells can grow is used. That is the reason myeloma cells have lost thepotential to secrete hypoxanthine - guanine - phosphoribosyl transferase (HGPRT), a chemical substance important for the salvage synthesis of nucleic acids. By uncovering cells to aminopterin (counterpart of folic acid, which hampers Dihydrofolate reductase, DHFR), they are impotent to use the de novo procedure and become completely auxotrophic for nucleic acids prerequisite supplementation to survive.

HAT is the selective culture medium because it contains hypoxanthine, aminopterin and thymidine. It is particular for



Different stages in production of monoclonal antibody

Stage 1: Immunization of Mice and Selection of Mouse Donors for Production of Hybridoma Cells

Mice are vaccinated with an antigen that is ready for infusion either by mixing the antigen with adjuvants or by integrating a gel slice that inhibits the antigen. Intact cells, whole membranes, and microorganisms are occasionally used as antigens. Mostly, mice are used to develop the required antibodies. Normally, mice are vaccinated every 2-3 weeks but the immunization protocols differ among examiners. When desirable amount of antibody titer is reached in serum, immunized mice are sacrificed and the spleen extracted to use as a source of cells for fusion with myeloma cells.

fused hybridoma cells. Unfused myeloma cells lack HGPRT so they cannot grow and accordingly cannot replicate their DNA. Uncombine spleen cells cannot grow endlessly because of theirrestricted life expectancy. Only fused hybrid cells, known as hybridomas, can grow regularly in the media because the spleen cell partner provides HGPRT and the myeloma partner has property that make it undying like a cancer cell. This mixture of cells is then diluted and clones are developed from single parent cells on separated small wells. The antibodies synthesized by the numerous clones are then assessed for their potential to bind to the antigen (with an assaylike ELISA or Antigen Microarray Assay) or immuno-dot blot. The most beneficial and stable clone is then designated for future use.

The hybridomas can be multiplyendlessly in a desirable cell culture medium. They can also be infused into mice/rabbit (in the peritoneal cavity, surrounding the gut). There, they give rise tomalignantexcreting, antibody-rich fluid called ascites fluid. The medium has toimproveduring in-vitro selection to further help hybridoma growth. This can be accomplished by the utilizationof a layer of feeder fibrocyte cells or supplement medium such as briclone. Culture-medium nourished by macrophages can also be used. Production in cell culture whichgenerallyrepresents ascites technique, is traumatic to the animal.

Stage 2: Screening of Mice for Antibody Production

After a few weeks of vaccination, blood samples are acquired from mice for estimation of serum antibodies. Serum antibody titer is resolved with different techniques, for exampleflow cytometry and enzyme-linked immunosorbent assay (ELISA). If the antibody concentration is high, cell fusion can be carried out. If the concentration is too low, mice can bestimulated until a satisfactory reactionis achieved, as decided by continuous blood sampling. When the antibody titer issufficiently high, mice are normally stimulatedby infusing antigen without adjuvant intravenouslyor intraperitoneally three days before fusion but two weeks after the previous immunization. Then the mice are sacrificed and their spleens extracted for in vitro hybridoma cell production.

Stage 3: Preparation of Myeloma Cells

Fusing antibody-producing spleen cells, which possess restricted life expectancy, with cells derived from an everlasting tumor of lymphocytes (myeloma) results in a hybridoma that is efficient forimmeasurable growth. Myeloma cells are undying cells that are rearedwith 8-azaguanine to ensure their reactivityto the HAT selection medium used after cell fusion. Seven days before cell fusion, myeloma cells are grown in 8-azaguanine. Cells must have high feasibility and rapid development. The HAT medium permits only the fused cells to remain in culture.

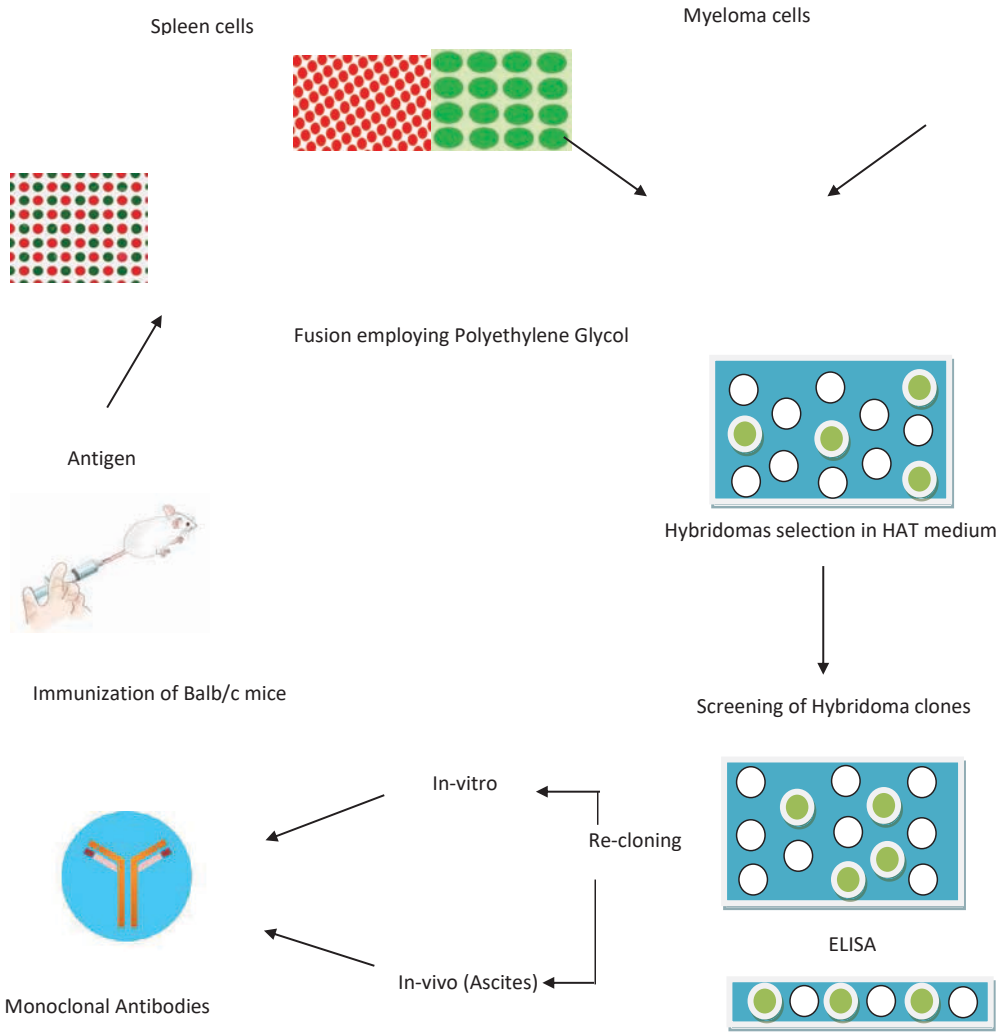


Fig. 1. A schematic illustration of the production of monoclonal antibodies

Stage 4: Fusion of Myeloma Cells with Immune Spleen Cells

Single spleen cells removed from the vaccinated mouse are intertwinedwith the recently prepared myeloma cells. Fusion is completed by co-centrifuging newly harvested spleen cells and myeloma cells in polyethylene glycol, a chemical substance that favors cell membranes to fuse. As discussed in step 3, only fused cells will flourish in the special selective medium. The cells are then circulated to 96 well plates having feeder cells got from saline peritoneal washes of mice. Feeder cells are used to supply growth factors that stimulate development of the hybridoma cells. Commercial production that arise from the collection of media favoring the growth of cultured cells and carry growth factors are available that may be used instead of mouse-derived feeder cells. It is also conceivable to utilizemurine bone marrow acquired macrophages as feeder cells.

Stage 5: Cloning of Hybridoma Cell Lines by "Limiting Dilution" and Stabilization of Clones by Ascites Production

In this stage new, small groups of hybridoma cells from the 96 well plates can be grown in tissue culture followed by antigen binding selection or propagate by the mouse ascites method with cloning period of time in the future. Cloning by restricting dilution at this time ensures that most of wells containsnot more than a single clone. Extensive judgment is important at this stageto select hybridomas capable of development versus collapse of the cell fusion product due to under population or insufficient in vitro developmentat high dilution.

Advantages of Monoclonal antibodies:

- Highly specific recognition of only one epitope of an antigen
- Immortal hybridoma cell lines can deliver unlimited amounts of antibodies
- High consistency among tests
- Minimal cross-reactivity
- Excellent for affinity purification

Disadvantages of Monoclonal antibodies:

- Developing a monoclonal requires high technical skills and takes time.
- They can produce lot of specific antibodies but may be too specific to detect over a range of species.
- Vulnerable to the change of epitope. Even a slight change in conformation prompt drastically decreasedbinding capacity.

Application of Monoclonal Antibodies in Aquaculture

Monoclonal antibodies (MAbs) are being utilized in pathogen classification, disease diagnosis, epidemiological investigation and improvement of vaccines. Because of their specific-nature, monoclonal antibodies are better than the polyclonal antibodies of regular methods and possibly more powerful than contemporary medicationsused for fighting disease. Drugs often attack the body's own personal cellsin addition to the foreign particle, causing side effects such as itching and nausea. Monoclonal antibodies will bind to only the particular target molecule, without any undesirable side effects. When monoclonal antibodies for a desirable substance have been produced, they can be utilized to recognize the presence of this substance.

Table 1. The monoclonal antibodies are superior in a number of aspects to conventional polyclonal antisera

	Conventional antiserum	Monoclonal antibody
Determinant	Several	Single
Specificity	Variable with animal and bleed Partial cross-reactions with common determinants Seldom too specific	Standard Unexpected cross reactions may occur; May be too specific for requirements
Affinity	Variable with bleed	May be specific during cloning
Yield of useful antibody	Up to 1 mg/ml	Up to 100 mg/ml in tissue culture; up to 20 mg/ml in acidic fluid
Contaminating immunoglobulin	Up to 100%	None in culture; 10% in acidic fluid
Purity of antigen	Either pure antigen or serum absorption	Some degree of antigen purification desirable but not essential

Source: K.M Shankar et al., 2000

Monoclonal antibodies (MAbs) were developed against enterotoxin of *Vibrio cholerae*, a brackish water and estuarine bacterium which causes cholera. MAbs based ELISA have been used for studies of *Vibrio anguillarum* strains and for investigation of clinical cases of furunculosis (*Aeromonas salmonicidae*) and Enteric Red mouth (*Yersinia ruckeri*) in fish farms. MAbs are also used to investigate fish parasities. MAbs have been produced against pathogenic protozoans (*Cryptobia salmonsitica*, *Ceratomyxa shastia*, *Bonamia ostreae*, *Perkinsus maximus*) of shell fish. Monoclonal antibodies for EUS fungus *Aphanomyces invadans*, *A. hydrophila*, and white spot virus of shrimp have been developed and being utilized in diagnosis in India. Use of a MAbs against virus: - Identification of Infectious Pancreatic Necrosis virus (IPNV) by ELISA. ELISA could be used for the detection of different serotype of IPNV. Infectious hematopoietic necrosis (IHN), caused by IHN virus (IHNV), is a critical and acute epizootic among salmonid fish. MAbs against IHNV HV - 7601, were produced.

Table 2. Specificity and commercial availability of monoclonal antibodies for use in aquaculture

Specificity	Availability
Aeromonas salmonicida	Diag Xotics Inc*, 27 Cannon Road, Wilton CT 06897 USA
Renibacterium salmoninarum	Aquatic Diagnostics Ltd., Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK Diag Xotics Inc*, 27 Cannon Road, Wilton CT 06897 USA
Infectious Pancreatic necrosis Virus (IPNV)	Diag Xotics Inc*, 27 Cannon Road, Wilton CT 06897 USA Test-Line Ltd Clinical Diagnostics, Krizikova 70, 61200 Brno, Czech Republic*
White spot virus (WSV)	Diag Xotics Inc*, 27 Cannon Road, Wilton CT 06897 USA
Taura syndrome virus (TSV)	Diag Xotics Inc*, 27 Cannon Road, Wilton CT 06897 USA
Spring viraemia of carp virus (SVCV)	Test-Line Ltd Clinical Diagnostics, Krizikova 70, 612 00 Brno, Czech Republic*
Viral haemorrhagic Septicaemia virus (VHSV)	Test-Line Ltd Clinical Diagnostics, Krizikova 70, 612 00 Brno, Czech Republic*
Snakehead (*Channa striata*) IgM	Aquatic Diagnostics Ltd. Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
Catfish IgM (*Clarias sp.*)	Aquatic Diagnostics Ltd. Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK

Source: http://ecoursesonline.iasri.res.in/mod/page/view.php?id=46117

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Ultrasonic Wave against Pathogens in Aquaculture and its Various Applications in Fisheries

Highlight Points

- Ultrasound technology is an emerging field in commercial aquaculture having varieties of applications.
- The sonic wave of resonance frequency helps in the removal of pathogens, parasites, and harmful algal blooms from the aquatic ecosystem.
- Sonic technology is an eco-friendly method targeting only the pathogen without seriously affecting the fishes in the culture environment.

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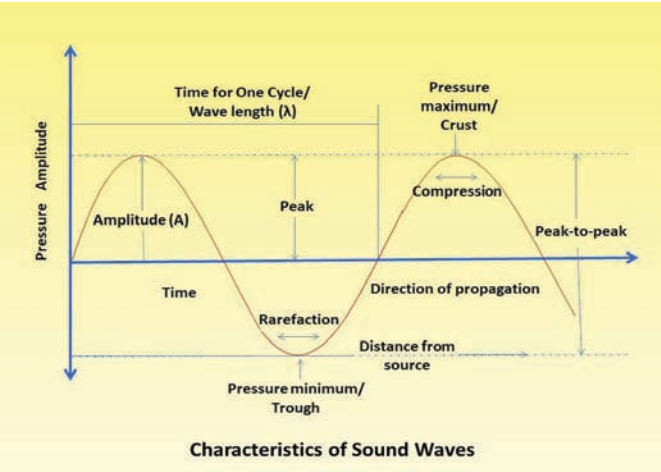
Abstract

From time immemorial, the use of energy, frequency, and vibrations have been put forth by many. But, its many angles of utilities are yet to be explored; one such exploration is the application of sound waves to treat diseases by the destruction of pathogen or causative agent of the disease in the aquatic environment. The vibration of parasite cells or a microbe is the concept of destruction which is done utilizing the resonance frequency of the particular organism. Each organism's core frequency is called the resonance frequency at which the cells will vibrate in its nature. When this resonance frequency is applied to the particular organism, its cells break, resulting in death and this kind of technology is gaining importance in aquaculture, especially against sea lice infection of cultured cold water fishes. Although ultrasound technology has been successfully utilized in various fields, including medicine, its uses are not restricted. This article explains the applications of ultrasound and other sonic technologies in fisheries and aquaculture.



Characteristics of Sound Waves

Introduction

As aquaculture intensifies, complications associated with management also emerge. Thus, to reduce and prevent circumstances such as the occurrence of diseases, low water quality, and harmful algal blooms, remedies are being sorted out. Most of the therapeutics used as remedial measures for eliminating these dreadful situations are having their side effects in the culture organism. For this reason, the arrival of eco-friendly as well as the culture organism-friendly method is being welcomed. The application of ultrasonic waves in aquaculture is one among them. Even though sonic wave technology is applied in many branches of science, in aquaculture, it is a new advent which is expected to have a significant scope. More research is needed in this field against fish pathogens. Although the use of SONAR for marine fishing and navigation had started early, the core application of sound waves in other aspects of fisheries, aquaculture, and fish processing is a lot. Such explorations can be achieved by combining the capacities of fisheries engineers and fisheries biologist. An integrated approach is needed in expanding the branch of acoustics in the aquaculture sector along with its already existing applications in marine fisheries.

Technologies utilizing sound waves are used for various applications such as interior characterization, repulsion of insects, seismic imaging, audio data technology, acoustic tweezers, acoustic levitation, doppler ultrasonography (DUSG), scanning near-field ultrasound holography (SNFUH), and high intensity focused ultrasound (HIFU). Spectral ultrasound imaging (SUSI) is used for structural property and composition analysis of tissues in animals using sound waves. In the fisheries sector, sonic technology has been utilized for parasite control, pond algae control, extraction of lipids from microalgae, 'SONAR' for fishing and navigation, ocean floor mapping using interferometric synthetic aperture sonar, acoustic density estimation of dense fish shoals, and sonophoresis for enhanced vaccine uptake. The ultrasonic treatment has been found to increase the yield of collagen extracted from the skin of fishes such as sea bass. Sonic wave technology has been shown to reduce the egg hatching time of marine fish. This technology also improves electricity generation in micro fuel cells. In the seaweed (*Gracilaria* sp) culture environment removal of fouling organisms and dirt from the algae is facilitated by sonic technology. Another important use of this technology is the ADDs (Acoustic Deterrent Devices), used for the avoidance of sea mammals in the fish cage culture provinces and alert for the presence of netting in the sea. Thus, sonic and ultrasonic methodologies are utilized in various aspects of fisheries and aquaculture.

What is an ultrasonic wave?

Sound is defined by Popper and Carlson (1998) as a density disturbance that propagates through a medium. 'Ultra' means high and 'sonic' denotes sound, which specifies the very high level of sound that's beyond the human hearing range and is above 20KHz. Naturally sound waves at this range are employed by some nocturnal creatures, marine animals, and insects. Examples include marine mammals such as whales, dolphins, orcas, porpoises, and other animals such as bats, rodents, and birds. This high-frequency sound wave is used in medicine to visualize the internal organs of the body or fetal imaging, and this process is called as Sonography.

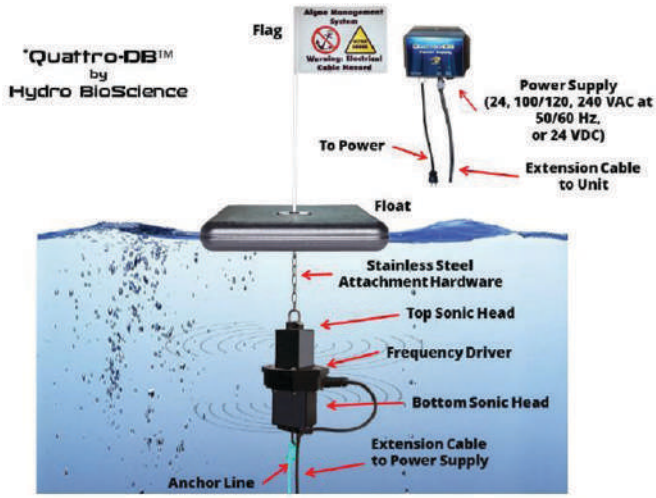


Illustration of Quattro DB ultrasound system

Usage principle

Though the ultrasound wave technology has various applications, utilizing it for pathogen removal in aquaculture systems is still not commercialized in India. Eliciting sound waves of any frequency without regulation in our area of interest will not ascertain the expected result. Finding out the resonant frequency is the fundamental principle of using the ultrasonic wave. For instance, consider targeting a pathogen or a parasite from a fish. The 'fish' and the 'pathogen attached along with the fish' are having different resonant frequencies. So targeting the pathogen needs only to find out its resonant frequency to solve the problem, which is quite tricky because of the uniqueness of the frequency from one organism to another. Once the resonant frequency is known, the other environmental factors affecting the process need to be addressed. Proper utilization of ultrasonic resonance frequency breaks the cells of the pathogen resulting in its death. The spreading of energy using the sound wave over massive areas results in the reduction of the power per area, which is called 'geometric scattering'. Hence the area of treatment should be near the source of the ultrasonic wave generator for better results. As the sound wave propagates, some energy is absorbed depending on the temperature, pressure, and salt content of the medium. The factors such as scattering, reflection, and deflection contribute to the weakening of the sound wave as the wave propagates to a more considerable distance.

Other criteria needed for consideration is the medium in which sound wave is used. Typicallysound waves travel fast and work fine in the liquid medium than air. Hence,the net effect will be more in liquid medium than air. Also, air can be considered as a significant barrier in the case of using an animal specimen for imaging using the ultrasonic wave. Usages of sonic wave need the consideration of several other factors which includes the variables such as frequency, intensity, beam dimension, and duration for effective utilization.

Bacterial control

Pathogens in aquaculture include all the organisms which cause diseases comprising the bacteria, fungus, virus, and parasites. For controlling the bacterial population, ultrasonic wave usage was demonstrated by Drakopoulou *et al*.,(2009) in which the deactivation of the gram-positive as well as the gram-negative bacteria was done at 24KHz. Utilizing this new approach, the bacterial population in the wastewater effluents in addition to aquaculture systems can be controlled. This approach provides the safe side of reducing the bacterial load, including the pathogens rather than using antibiotics. The usage of antibiotics once called the 'boom in medicine' is now losing its power because of antimicrobial resistance (AMR) occurrence among the pathogens. Also, there are strict regulations for using antibiotics in aquaculture. Considering all this, the need for developing new techniques as remedies is essential in the case of pathogenic microbes which cause diseases. For this reason, the ultrasonic wave technology can be utilized since its use in the water system does not harm the fish under culture rather affects the target pathogen against which the frequency is used.

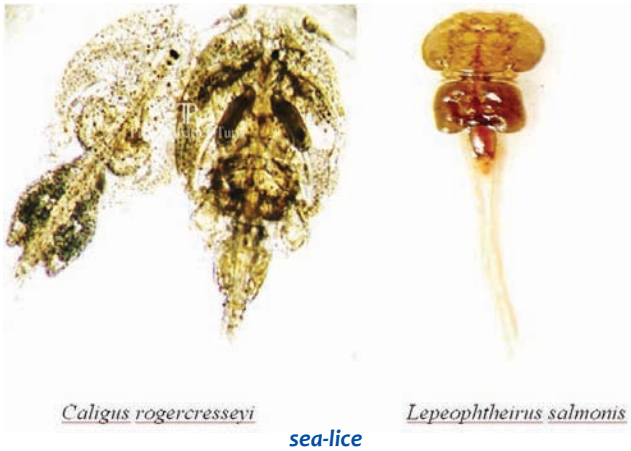
Reduction in the bacterial load occurs by using the pulsed sonicators of the resonant frequency. An increase in the power and treatment time results in the total coliform reduction in the water. Coliforms are considered as the indicators of faecal contamination in water, and their presence is undesirable in the drinking water. But to use sonication in an economically-friendly manner, the power usage can be reduced by increasing the pulsation off duration. Also, the bacterial load reduction was found to occur in log values among the bacterial species such as *Enterobacteraerogenes, Bacillus subtilis, Staphylococcusepidermidis*, with the use of ultrasound upon increasing the power. Besides the bacterium, *E. aerogenes* is found as more sensitive to the ultrasonic wave during its 'exponential growth stage', which is the faster multiplication stage of the bacteria. So this way of minimizing microorganism load can be done for yeast (*Aureobasidiumpullulans*) and bacteria that are present in the water. This inactivation is carried out by the production of free radicals and reactive oxygen species that damage the cell. The inactivation mechanism continues in the treated cells even after the sonication process is terminated. But there is a drawback in using this ultrasonic wave against capsulated bacteria as these bacteria are found to confer resistance to the sound wave. The capsules are external structures surrounding the bacteria comprising mucopolysaccharide which involve in various protective mechanisms of the bacteria. Hence, finding out the mechanism to disrupt the capsule of this kind of bacteria may aid in destruction.

Heckerman *et al.,* in 2010, have patented a system to disrupt pathogens using ultrasound. Wherein a computer-aided with the resonance frequency database of the pathogen is used to compare the average resonance frequency of the healthy cells surrounding the target material. But this ultrasound system is designed for treating targets in the cells and tissues of higher animals and not for application in the aquatic environment. However, this technique can be modified and adapted for the underwater system, for which invention is waiting to be made by the innovators.

Another approach of controlling the bacteria is combining the sonication with the traditional bacterial treatment process such as the use of antibiotics; wherein, the low dosage of the authorized antibacterial component is enough to act against the pathogen when used in combination with the quiet intensity sonication process. This process is useful against *Chlamydia*, planktonic bacteria, and bacterial biofilms. The low-intensity sonication alone is not effective against bacteria unless it is combined with an even low dose of antibiotics.

Parasite control

In temperate aquaculture, salmon farming is a profit yielding industry in some countries such as Norway. But the occurrence of parasitic infection is a common phenomenon in the cage culture provinces of the salmon farming industry. Sea lice are the ectoparasitic copepods coming under the family 'Caligidae'. They are found attached to the skin, gill, and fin of the fishes and feeds on the skin, mucous, and blood of the fishes. The lice create wounds in the fishes in the feeding area, thereby allowing the secondary pathogens to invade inside the fish through the scars. For controlling this parasitic infection, various chemicals are used, including cypermethrin, emamectin benzoate,teflubenzuron, hydrogen peroxide, dichlorvos, azamethiphos, and pyrethrum. But the use of chemicals will result in environmental impacts and other side effects in the farmed fishes. Also, the chemicals result in high treatment costs due to repetitive treatments. The Norwegian Food Safety Authority has set an upper limit of 0.5 sexually mature female lice per salmon as an acceptable limit in fish farms. When the limit exceeds, the treatment must be initiated in the culture system.



sea-lice

The Chilean sea lice (*Caligus rogercresseyi*) are the main parasite which corresponds to the loss of USD 300 million in the year 2009. This parasite is also found as a vector of transmitting Infectious Salmon Anaemia virus (ISA) among the fishes. Sea lice are attracted to light at the earlier life stage. By employing this strategy along with the sonic technology, a commercial sonicator is developed by a Chilean company called 'Usonic' which is created under CORFO (Chilean National agency of economic development). This technology results in a 30-50% reduction in sea lice load. The company 'Usonic Ltd' has got the Global Aquaculture Alliance's innovation award for its development of the method of controlling the sea lice in 2014.

Further, removal of salmon lice, *Lepeophtheirus salmonis* was tried in a small scale study in which the sound waves with the frequency of 9.3KHz elicit cavitation effects on the parasite along with the reduction in the parasitic load in the host animal. However, field application of ultrasonic waves for the removal of *Lepeophtheirus salmonis* is not assured. Thus the technology needs optimization for every kind and size of the target organism.

Steven Alevy in 2017 had patented a device for removing the sea lice from the atlantic salmon using ultrasonic waves. Wherein he designed a herding passage tube for salmon in which the transducers are fitted on the periphery of the tube. The tube is lined interiorly with stainless steel and exteriorly with the sound-absorbing material. This tube is used for removing sea lice from salmon during the normal herding process. Still, the method can also be employed in the cage cultured salmons wherein the fishes should

be fed in an enclosure, and white light should be used for attracting the parasitic sea lice along with the application of the ultrasound from the electronic sonicators which should be fitted at the inner periphery of the province. This method is encouraged in Indian cage culture systems for parasite removal.

Pond algae control

Nonchemical algal control measures are cheered in the aquaculture industry for which the sonic system is the best approach. It is a cost-effective approach and can be even utilized for larger ponds. The sonic technology can be applied for the eradication of harmful algal blooms caused by Cyanobacteria such as *Microcystis aerogenosa.* Algal cells contain small vacuoles of air inside for buoyancy which gets vibrated by the ultrasonic waves resulting in its damage. Vibrations damage the cell wall and result in the condensing of the cell contents at the centre of the cell by which the normal nutrient uptake is avoided. This method does not affect the fishes but can be employed for the control of geosmin in water since the microorganisms produce geosmin. Filamentous algae can be controlled, but the larger macrophytes such as 'chara' and 'duckweed' are resistant to the sonic waves. Algal coagulation removal can be improved by 12.4% when combined with ultrasonic treatment at 40KHz and 60W for 15s. Algal removal commercial sonicators are available in the market, for example, Quattro DB ultrasonic algae control system, Mezzo ultrasonic algae control system, and ASMP ultrasonic sonic system. One of the ultrasonic algae and bio-organisms controlling apparatus is patented by Antonio Trigiani, U.S. This apparatus includes a power unit and a transducer that has a sonic head that can radiate from multiple angles. The frequencies used in this apparatus include the critical structural resonant frequency for each microorganism that is to be controlled.

Prevention of fouling:

The marine ships and the water inlet pipes are in continuous contact with the aquatic environment resulting in the provision of substrate for fouling organisms. Fouling poses a severe threat to the small diameter pipes as they clog the inlet or outlet system by the assemblage of the macrofauna such as the mussels. For the avoidance of fouling, ultrasound waves were investigated by some researchers in which pulses generated for every 45 seconds significantly reduced fouling by zebra mussel up to 23m from the transducer. Usage of 200 W power transducer and 17-30KHz frequency range resulted in a marked reduction in fouling near the transducer. However, there are only a few studies with little consistent reporting when it comes to the practical use of ultrasound on ships (Legg et al., 2015). The product USAF (Ultrasonic Anti Fouling System) is a commercial ultrasound-based system to prevent fouling on boats. The product is manufactured and marketed by Luykx Ultrasound2 in the Netherlands. The manufacturer claims that the transducer creates cavitation bubbles in the water in the immediate vicinity and when these bubbles collapse generate shock waves that "scare away" scavengers and other organisms.

Control and modification of fish behaviour

Fishes use the sense organs such as the ear (otolith) and the lateral line system for detecting the vibrations in the water. The man-made sounds affect the behaviour of the fishes. Utilizing acoustic signals, modification of the action of the fishes was tried at the laboratory. Limited success has been achieved in the experimental change of behaviour of clupeids *Alosa pseudoharengus* and American shad to prevent them from entering into turbine intakes at dams. Yellow perch (*Perca flavences*) and pumpkin seed (*Lepomis gibbosus*) adults were prevented from entering the inlet of the Nuclear Generation station using pneumatic poppers (which doesn't use ultrasound). Some species of clupeids can detect ultrasound such as alosids. In a study at the Vernon hydroelectric station, ultrasound helps in moving fish away from turbine intake. The acoustic guidance system can be used for migrating fishes such as eels for preventing them from entering hazardous places. Hence, more understanding about the behaviour of fish against the ultrasound will help in the future for avoiding the fishes being entered into turbines and other inlet sources of commercial application.

Non-invasive method for inducing fish growth

The effects of sound on fish growth performance were evaluated on ornamental fishes which showed good growth results upon playing the binaural beat complexes for 90-270 min. The binaural beat treatment also showed high feed efficiency in fishes. The sonic waves having slightly different frequencies are called binaural beats with both the frequencies lower than 1500 Hz, with the difference between them less than 35 Hz. Hence, binaural beats can be used in aquaculture for enhanced fish production.

Acoustic telemetry tags

It is one of the tracking applications in fisheries utilizing sound waves. The labels are fitted with the fish, and the fishes are released in the aquatic environment. 'Acoustic telemetry tags' emits sonic waves which help in the tracking and monitoring of the behaviour of the fishes. The acoustic tags emit pulses of signals which in turn are received by the hydrophone receiver. These tags are more useful in studying the biology of the fish species, which are mainly migrants. The different development stages of the fish can be tagged to check the migration of the organism during the particular period of its lifecycle. The activity and movement levels of American lobster*Homarus americanus* in natural habitatis quantified using ultrasonic telemetry in Canada. Similarly, this technique can be employed for tagging Indian migrant fishes and endangered species to study deeply about their life cycle.

Extraction from algae

Algae cells serve as a good source of lipid and various other components. Ultrasound technology helps in the extraction of lipids from the algal cells. Among the various methods utilized for algal lipid extraction, the 'Bligh and Dyer method' assisted by ultrasound was found to contain the highest yield from the algae *C. vulgaris* and is about 52.5% w/w. The various extraction processes from the macroalgae can also be improved with the combination of

the ultrasound with the existing methods. The extraction of phycobiliproteins from the marine red macroalgae *Gelidium pusillum* was enhanced when the maceration of the algae is combined with the ultrasonication treatment. Usage of 300W ultrasound-assisted extraction (UAE) with the extraction time of 38.3 min was found to give the highest yield of taurine from *Porphyra yesoensis*.

For increased biogas production, sonic technology can be a powerful tool. At higher applied energy, the ultrasound treated cells show a higher rate of disintegration, resulting in increased biogas production. The ultrasound pre-treated *Hydrodictyon reticulatum* show increased biogas production by methane potential test than the non-treated one. Some other applications of ultrasound in fish processing technology include filtration, defoaming, depolymerization, drying, defrosting, freezing (by power-variable ultrasonic waves in saltwater immersion), and homogenization. Ultrasound treatment is found to accelerate the rehydration of dried sea cucumber in the processing industry. Thus various usage methods have been developed in the fish processing industry, enabling sonic technology.

Conclusion:

Ultrasonic technology is a promising alternative for therapeutics and antimicrobials. But the future extent of commercialization is not fully known even though the commercial algal control and sea lice removal equipment are available. Moreover, this technology does not harm the fish and has no negative effect, and reduces the physical handling of the fish for parasite removal. It is an environment-friendly method, hence ecologically advantageous. Though the technology applies for parasite and pathogen removal, the other mentioned uses of the technology are not limited, and innovations can be made utilizing the principle of the technology. But more research is needed in this sector for field application in tropical countries like India. The research trials can be initiated in the cage culture provinces in India for effective parasite removal from the systems.

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More References can be provided on request.

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Application of Monoclonal Antibodies in Aquaculture

Conclusion

Monoclonal antibodies present an appealing alternative for the improvement of new treatment methods against a wide variety of common diseases, because of their specificity, effectiveness and flexibility.Understanding a pathogen itself is a main step in disease. There is lot of scope for the application of MAbsin aquatic animal health management worldwide. At present, MAbs of pathogenic protozoans, *Aphanomyces invadans* associated with EUS and white spot syndrome virus (WSSV) are being developed mainly for diagnosis and epidemiological examination. Apart from the impact on laboratory diagnostics, monoclonal antibodies illustrate a highly efficient treatment tool. There is a need to alter conventional treatment methods and shift interest towardsMAbs to avoid or minimize pain and suffering by the animals. In vitro techniques for the development of monoclonal antibodies should be embraced as the standardroutine method.The manipulation of antibodies via binding other molecules or designing new antibody fragments opens the door to a wide range of possible applications in medicine.

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