ISAAHE

International Symposium on Aquatic Animal Health and Epidemiology for Sustainable Asian Aquaculture

April 20-21, 2017

ICAR-National Bureau of Fish Genetic Resources, Lucknow, India

सत्यमेव जयते

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ASIAN FISHERIES SOCIETY FOUNDED 1984 Indian Branch

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PREFACE

Sustainable growth of Asian aquaculture is important for livelihood and nutritional security in the region and at the same time for global availability of fish, a health food for every strata of human society, from resource poor to rich. Currently, about 90 percent of the global aquaculture production is contributed by Asia and the sector is growing annually at the rate of approximately 9 percent. The growth has largely been due to intensification and significant contribution from international movement of aquatic animals particularly non-native species. However, long-term sustainability of this intensification is under question due to various concerns such as finite availability of critical inputs, environmental impacts, genetic erosion, lack of use of improved varieties, failure to implement biosecurity measures, emergence of new diseases and spread of existing pathogens to new areas etc. Some of the pathogens, which have been responsible for huge losses in Asian aquaculture, include White Spot Syndrome Virus, *Enterocytozoon hepatopenaei*, *Macrobrachium rosenbergii* nodavirus and *Aphanomyces invadans*. Importantly, the catastrophic losses due to disease outbreaks are affecting mainly small scale rural farmers, who constitute about 80 percent aquaculture producers in Asia. The socio-economic impacts of the diseases are further aggravated by lack of preparedness and response to disease outbreaks. A number of risk factors determine and influence the frequency and distribution of disease and their causes in a population. The knowledge on risk factors can improve the ability of aquatic animal surveillance systems for early detection and rapid response to pathogens.

Keeping the above in consideration, the interdisciplinary experts consultation through this International Symposium during April 20-21, 2017 is very important. The Symposium covers presentations from renowned International and National Experts on Aquatic Animal Epidemiology and other relevant disciplines and will address a range of risk factors which catalyse horizontal spread of diseases, emergence of new pathogens, spread of transboundary pathogens, increased disease susceptibility and thus strengthening surveillance system especially in the Indian context.

I, on behalf of ISAAHE and ICAR-National Bureau of Fish Genetic Resources, Lucknow extend a warm and hearty welcome to all the participants. I would like to place on record my sincere thanks and gratitude to Shri Devendra Chaudhry, Secretary, DADF, Ministry of Agriculture and Farmers Welfare, Govt. of India for his continued support and guidance for the National Fish Disease Surveillance Programme. I am profoundly indebted to Dr. T. Mohapatra, Secretary, DARE and Director General, ICAR, New Delhi for his sincere advice and continued guidance. It is indeed a great opportunity for me to acknowledge my gratefulness for the unrelenting support and guidance of Sh. Aditya Kumar Joshi, Joint Secretary (Fisheries), DADF as the Chairman of Technical Advisory Committee of the National Surveillance Programme for Aquatic Animal Diseases and Chief Executive, National Fisheries Development Board for his constant support and encouragement. I am grateful to Dr. J.K. Jena, DDG (Fy.) and Coordinator, NSPAAD for his unstinted guidance. The continued help and cooperation provided by Dr. P. Paul Pandian, Fisheries Development Commissioner, DADF is thankfully acknowledged. I would like to convey my sincere thanks to Dr. B.K. Chand, Executive Director, NFDB and Shri I. A. Siddiqui, Assistant Commissioner (Fisheries), DADF for their constant support. My sincere thanks are also due to all the experts from India and abroad for kindly accepting our invitation as keynote speakers. I am also thankful to all the Directors of ICAR Fisheries Research Institutes for their continued support and cooperation all the time. I express heartfelt thanks to all my colleagues at ICAR-NBFGR for their untiring efforts and support. I sincerely hope that the symposium would be of immense benefit to the participants working in the area of aquatic animal health .

Kuldeep K. Lal
Convener, ISAAHE

राधा मोहन सिंह
RADHA MOHAN SINGH

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सत्यमेव जयते

कृषि एवं किसान कल्याण मंत्री
भारत सरकार
MINISTER OF AGRICULTURE
& FARMERS WELFARE
GOVERNMENT OF INDIA

12 APR 2017

MESSAGE

Fisheries is considered to be a sunrise sector for meeting the growing demand of quality animal protein and providing livelihood to large number of people. There has been almost 15 fold increase in fish production, from 7.5 lakh tonne in 1950-51 to 107.95 lakh tonne during 2015-16. India is the second largest fish producing nation in the world. Recently, Blue Revolution, the Neel Kranti Mission initiated by the Hon'ble Prime Minister has the vision to achieve economic prosperity of fishers and fish farmers as well as contribute towards food and nutritional security through full utilization of water resources for fisheries development in a sustainable manner. However, one of the major threats to the sustainability of the sector is expected to be from new emerging pathogens as well as spread of endemic diseases.

I sincerely hope that the International Symposium on Aquatic Animal Health and Epidemiology for Sustainable Asian Aquaculture would provide a stimulating and appropriate platform to the aquatic animal health experts, researchers and students for deliberations on a broad range of issues concerning the aquatic animal health and come out with strategies that can help in minimizing the risks of disease outbreaks.

I send my greetings and good wishes for the success of the Symposium.

Radha Mohan Singh
(RADHA MOHAN SINGH)

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सुदर्शन भगत
SUDARSHAN BHAGAT

D.O.No. 815/VIP/MoS(A&FW-SB)/Message

कृषि एवं किसान कल्याण
राज्य मंत्री
भारत सरकार
MINISTER OF STATE
FOR AGRICULTURE &
FARMERS WELFARE
GOVERNMENT OF INDIA

MESSAGE

It gives me a great pleasure to know that with ICAR-National Bureau of Fish Genetic Resources, Lucknow is organizing an International Symposium on Aquatic Animal Health and Epidemiology for Sustainable Asian Aquaculture during April 20-21, 2017.

India is the second largest producer of fish in the world today and the production has reached over 10.0 million tonnes which is about 15 fold increase over the post independence fish production. Fish is being recognized as a safe and quality animal protein, the demand is increasing day by day. As, horizontal expansion, even up to the level of 100% utilization of resources, may not be sufficient to cater the fish demand in the coming days, the production can only be increased through be vertical expansion through intensification and diversification. So, it is essential that aquaculture production is sustainably enhanced. However, aquatic animal diseases are one of the major hurdles for its sustainability and management of the diseases is considered a challenge. I am sure the outcome of the program would be useful for developing strategies for management of aquatic animal diseases. Further, climate change, increase in anthropogenic pressures, limited water sources add to this challenge and highlight the importance of epidemiological studies.

I wish the souvenir for a grand success.

(Sudarshan Bhagat)

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एस. एस. अहलुवालिया
S. S. AHLUWALIA

कृषि एवं किसान कल्याण और
संसदीय कार्य राज्य मंत्री
भारत सरकार
MINISTER OF STATE FOR AGRICULTURE
& FARMERS WELFARE AND
PARLIAMENTARY AFFAIRS
GOVERNMENT OF INDIA

सत्यमेव जयते

10 APR 2017

MESSAGE

It gives me immense pleasure to know that Aquatic Biodiversity Conservation Society, in collaboration with ICAR-National Bureau of Fish Genetic Resources (NBFGR), Lucknow and National Surveillance Programme for Aquatic Diseases in organizing an International Symposium on Aquatic Health and Epidemiology for Sustainable Asian Aquaculture during April 20-22, 2017.

Asian Aquaculture is the highest contributor to the global aquaculture production and the sector is growing at faster rate. The growth has largely been due to intensification of aquaculture and significant contribution from non-native species. However, sustainability of this sector is under threat due to various concerns and the some of the important concerns are failure to implement biosecurity measures and emergence of new diseases and spread of existing pathogens to new areas. This underlines the importance of epidemiological approaches to investigate the disease outbreaks.

I am glad to know that ICAR-NBFGR has identified Aquatic Animal Health and Epidemiology as the theme of the Symposium and I am sure that the two days symposium will address the concerns related to Aquatic Animal Health Management and help in developing strategies for disease control.

I wish the Symposium all the success.

(S.S. Ahluwalia)

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एफ एन ए, एफ एन ए एस सी, एफ एन ए ए एस
सचिव एवं महानिदेशक

TRILOCHAN MOHAPATRA, Ph.D.
FNA, FNASc, FNAAS
SECRETARY & DIRECTOR GENERAL

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MESSAGE

I am happy to know that an International Symposium on Aquatic Animal Health and Epidemiology for Sustainable Asian Aquaculture is being organized during April 20-21, 2017 by Aquatic Biodiversity Conservation Society, India in collaboration with ICAR-National Bureau of Fish Genetic Resources, Lucknow and National Surveillance Programme for Aquatic Animal Diseases, Government of India.

With food security being a primary concern for the country, aquaculture sector has the potential to play an important role by providing quality animal protein for the growing population, besides livelihoods for the people. For sustainable growth of the aquaculture sectors, management of diseases is crucial to avoid the production losses. I am sure that the Symposium will deliberate on a range of risk factors and their practical implications for progressive aquaculture. I sincerely look forward to useful recommendations to help sustainability in this sector.

I wish the Symposium, a great success.

(T. MOHAPATRA)

Dated the 13th April, 2017
New Delhi

देवेन्द्र चौधरी
Devendra Chaudhry, IAS
सचिव
SECRETARY

सत्यमेव जयते
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कृषि एवं किसान कल्याण मंत्रालय
पशुपालन, डेयरी एवं मत्स्यपालन विभाग
कृषि भवन, नई दिल्ली–110001
Government of India
Ministry of Agriculture & Farmers Welfare
Department of Animal Husbandry, Dairying & Fisheries
Krishi Bhawan, New Delhi-110001

18th April 2017

MESSAGE

I am delighted to know that the Aquatic Biodiversity Conservation Society is organising an International Symposium on 'Aquatic Animal Health and Epidemiology for Sustainable Asian Aquaculture' in collaboration with the National Bureau of Fish Genetic Resources (NBFGR), ICAR during 20th & 21st April, 2017 in Lucknow, Uttar Pradesh. I am happy to know that the NBFGR is making efforts to bring together all the stakeholders involved in the National Surveillance Programme for Aquatic Animal Disease for the development of fisheries sector and to promote aquaculture development which is the need of the hour.

Fisheries and aquaculture sector has been witnessing an impressive growth rate during the last decade. Considering the initiatives, both on scientific and research activities made by the NBFGR, they deserve compliments for coordinating and organising such events for the overall development of fisheries sector.

I wish that the activities proposed during the International Symposium are fully utilized by the stakeholders to enhance the understanding on various issues of the fisheries sector and get suitable advice and guidance from the Scientists and Experts in the field.

I congratulate the Director, NBFGR and his team and the authorities of organizations concerned for organising this International Symposium and I wish the event a grand success.

(Devendra Chaudhry)

भा.कृ.अनु.प.
ICAR
डॉ. जे. के. जेना
उप महानिदेशक (मत्स्य विज्ञान)
Dr. J. K. Jena
Deputy Director General (Fisheries Science)

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MESSAGE

Aquaculture has been one of the fast growing food-producing sectors in the world during last four decades. A major share of this production comes from the developing world where fish is an important part of the diet. As production from capture fisheries is stagnating, there has been increased emphasis on aquaculture over these years, and in this endeavour importance of aquaculture has been highly significant towards food security, livelihoods and human nutrition.

One of the major constraints to aquaculture production is the losses due to disease outbreaks. The sector has faced significant problems and heavy economic losses due to disease outbreaks over the last three decades. Disease outbreak is outcome of complex interaction involving environmental factors, health condition of the stocks, presence of an infectious agent and/or poor management practices. Controlling aquatic animal diseases, therefore, is not easy. The use of sound epidemiological principles, and logical and science-based approach to identify and manage risks can significantly improve health management.

I am happy to know that ICAR-National Bureau of Fish Genetic Resources, Lucknow, India is organizing an International Symposium on "Aquatic Animal Health and Epidemiology for Sustainable Asian Aquaculture" at Lucknow during April 20-21, 2017 in collaboration with Aquatic Biodiversity Conservation Society, India and National Surveillance Programme for Aquatic Animal Diseases. I am confident that the Symposium would provide an appropriate platform for scholarly interactions for the researchers and policy makers associated in fish health management, and developing management strategies and action plans for combating the present and emerging issues of fish diseases in the region.

I wish the event great success.

17/4/2017

(J. K. Jena)

सत्यमेव जयते

संयुक्त सचिव
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Joint Secretary
Government of India
Ministry of Agriculture & Farmers Welfare
Department of Animal Husbandry, Diarying & Fisheries
Krishi Bhawan, New Delhi-110001

MESSAGE

I am pleased to know that the Aquatic Biodiversity Conservation Society, India is organizing an International Symposium on Aquatic Animal Health and Epidemiology for Sustainable Asian Aquaculture during April 20-21, 2017, in collaboration with ICAR-National Bureau of Fish Genetic Resources, Lucknow and the National Surveillance Programme for Aquatic Animal Diseases.

Aquaculture sector in the country has shown a phenomenal growth of 6-7% per year, since the last two decades and is providing livelihood to a large number of fish farmers. Importantly, the sector is considered to be a potential supplier of quality animal protein in future. However, diseases have been recognized as major constraint to the sustainability of the sector and a number of risk factors are responsible for the spread and/or emergence of pathogens. Knowledge of risk factors can greatly help in developing strategies for management of diseases.

I am happy to note that this symposium aims to bring together leading aquatic animal epidemiology and health experts, scientists, research scholars and students to exchange and share their experiences. I am sure the two days symposium will come up with implementable recommendations which would help in mitigating the risk factors, strengthening the ongoing surveillance programme and thereby, minimizing the losses due to diseases in the country.

I sincerely wish the symposium a grand success.

(Aditya Kumar Joshi)
Joint Secretary, Fisheries

Contents

Souvenir Article

Executive Summary

Epidemiological Principles for Surveillance Design and Implementation

Hammell, K. L.*

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Design specifics of surveillance programs differ depending upon the intended purpose, but the principles remain constant. For authorities responsible for international trade, demonstrating absence of infection is the most common purpose for establishing rigorous surveillance programs. However, similar surveillance principles apply to domestic disease control in which the capacity to describe the spatial and temporal distribution of infection and disease informs control efforts. The systematic ongoing collection, collation and analysis of animal health information provides the evidence to support declarations of infection freedom and also to optimize decisions to reduce the transmission of a recently introduced pathogen or an already established, endemic pathogen.

Surveillance programs must balance resource limits versus information integrity. Effective disease surveillance can encompass simple passive systems that provide basic health information, particularly useful for some situations of demonstrating disease absence, to comprehensive, targeted systems for rapid detection of changing infection levels.

Using sound epidemiological principles to inform the design and implementation of surveillance programs will increase the probability of correct conclusions about infection absence or distribution patterns. This presentation will provide an introduction to the important aspects of infection transmission pathways (e.g. live animal movements), risk factors for introduction and spread (e.g. farm management practices leading to increased probability of exposure), probability of detection based on diagnostic testing (including diagnostic sensitivity and specificity), selection bias (e.g. increasing the probability of incorporating an infected animal or group using risk-based sampling), and quantifying the uncertainty of conclusions. Concepts of defining the population of interest and what constitutes a case, or suspect case, will also be discussed as crucial aspects of appropriate surveillance design.

Evidence-based decisions for national or regional aquatic animal disease control depends on the most effective design and interpretation of surveillance programs adapted to the environmental conditions and disease states of interest. The probability of the surveillance program detecting an emerging disease is affected by transmission properties and host responses for the pathogen, exposure patterns, and the capacity for detection. However, it can also be susceptible to under-reporting and conflicts with end-users who may wish to avoid the economic consequences of enforced control actions, particularly with potentially false positive testing results. Selected aspects of these influences on the implementation of surveillance programs will be discussed.

Farmer-based Syndromic Surveillance: A Practical Approach

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The human aspects of surveillance represent an important knowledge gap in animal disease surveillance. The fast pace of aquaculture development, the volume and complexity of the network of global transportation of aquatic species, fish and shellfish products pose a challenge to epidemiologist and their traditional tools of estimating the frequency, distribution and determinants of disease in population.

Information and communication technology, cheap and wifi linked sensors and the data mining and classification power of supervised and unsupervised machine learning such as Support Vector Machine represent just the beginning of technological and data processing capacity, the full extent of which is impossible to grasp for a society that has moved from slates, chalk and penfriends to Siri, Google and Facebook in less than a century.

Is the technological revolution the panacea to epidemiolocal intelligence or are there aspects of the interaction of people with these technologies, particularly in surveillance, that we are missing?

The central argument in this presentation is that if data collection for epidemiological monitoring is about technology, surveillance itself is about people.

Aquatic Epidemiology in Developing Countries: Using Data and Collaboration to Drive the Aquaculture Sector

Corsin, F.*

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Health and diseases is arguably the most critical challenge the aquaculture sector faces. Diseases have always challenged the development of the sector. Although the sector generally bounces back after an outbreak and considerable progress has been made in pathogen and disease detection, it does not seem to be any more prepared to deal with diseases than it was a couple of decades ago.

In fact, similarly to previous diseases, the Early Mortality Syndrome (EMS) outbreaks in shrimp in Asia and Latin America have led to crop failures for several hundreds of thousands of producers, resulting in global price and supply volatility of farmed shrimp. In subsectors that are not affected by major disease outbreaks the volatility in survival ranges in the two-digit percentage figures for which weather and seed quality are generally blamed, often without concrete evidence. Agrochemicals (e.g. antibiotics) use is still prevalent in large segments of the industry, arguably driven by a general lack of knowledge on the application and effectiveness of such treatments against the emerging disease problems. This often leads to misuse of these substances, which may affect food safety and market access of aquaculture products. As food safety is the top priority for seafood buyers, aquatic animal health management is a critical issue to address.

The application of epidemiological tools to the understanding and management of aquatic animal diseases is a relatively recent development in the field of epidemiology. The approach is at best a couple of decades old and was first applied to salmonids, where the structure of populations and the socio-economic status of the countries and farming communities was more suitable to a population-based, data-driven approach to management. Aquaculture in developing countries, especially shrimp farming, offers several difficulties to the application of epidemiological tools:

- Farms are generally small-scale, which is generally associated with a limited degree of literacy, limited access to resources (including technical and financial) and count in the millions, each with their own management, hence bringing considerable diversity to the farming system

- Farms are scattered over a relatively large number of countries, over large areas and their accessibility if often limited
- Developing countries have often more limited resources to invest in the sector and in the adoption of a more data-driven approach
- A great deal of the products are consumed by national and regional markets that give less value to voluntary certification, often based on large quantities of data being collected and used

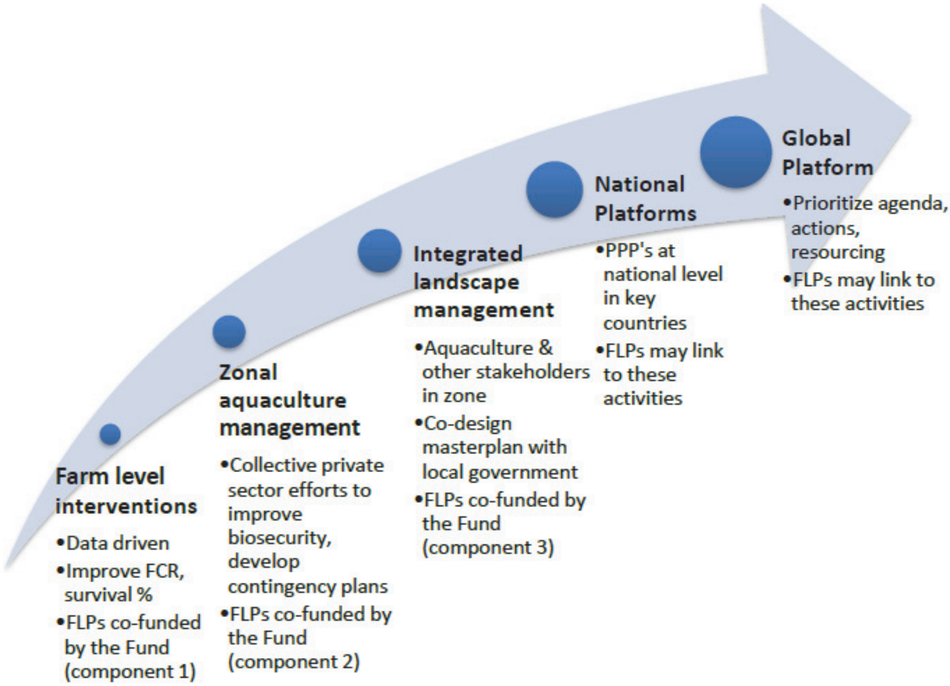
For the above reasons collection of population data to **understand** how diseases behave in fish populations is often very difficult. The above reasons are also associated with similar, if not greater, challenges towards the application of epidemiological tools to **control** diseases.

IDH the Sustainable Trade Initiative, which was created in 2008, has a short but intense history in aquaculture:

- In 2010, together with WWF, it created the Aquaculture Stewardship Council (ASC), now a leading sustainability certification program and the only aquaculture scheme to be a member of the International Social and Environmental Accreditation and Labelling (ISEAL) Alliance
- In 2012 it partnered with the Vietnamese government, producers, associations and WWF to establish the ASC accelerator, which led to the ASC certification of more than 20 percent of the Vietnamese pangasius production. Through this step the sector could preserve its position in key markets e.g. Europe
- In 2013 IDH launched its Farmers In Transition (FIT) Fund which, among others, supported the upgrading towards sustainability of about 200,000 metric tons of shrimp produced globally. The FIT Fund has been operating to this day.

A similar track record can be found for the 10 other sectors in which IDH operates (e.g. coffee, cotton, tea, cocoa, etc.). In 2017 IDH began rolling out its plan to address sustainability by focusing on the biggest challenges in the aquaculture sector, i.e. addressing specifically health and disease management, primarily in developing countries and in 3 species groups: shrimp, pangasius and tilapia. The program focuses its actions on key challenges of the sector: collaboration and data use.

Collaboration is addressed through the strengthening of global and national level public-private platforms that allow the identification of the sector priorities, required actions and the alignment of resources to support those actions. The field level collaboration and the promotion of data use is largely delivered through the FIT Fund, which supports focused Field Level Projects (FLPs) implemented through partnership and co-financing with the private sector and in alignment with the public sector.



The FIT Fund can co-invest in field level projects (FLPs) at various levels ranging from supporting private sector companies to adopt a data-driven approach (e.g. making better use of data collected e.g. for certification purposes) to improving farm efficiency (component 1), to improving disease management amongst aquaculture producers by developing zonal management regimes (component 2), to full-fledged landscape integration of zonal management with aquaculture producers and other public and private sector actors in the landscape with the aim to improve health and disease management for the area (component 3). These activities in the field can then be linked to platforms at national (component 4) or global level (component 5) as to strengthen collaborative efforts.

The potential to engage in a more structured way Indian producers and the Indian government into this multi-stakeholder global effort will be presented and discussed.

Policy Framework for Preventing Spread of Diseases in India

Ponniah, A. G.[1*], Sood, N.[2] and Pradhan, P. K.[2]

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Policies aimed at preventing spread of endemic diseases and introduction of exotic diseases in India will go a long way in boosting aquaculture production. Identification of disease risks associated with import of live and frozen aquatic animal products can help plan risk management measures. Along with risk analysis, contingency planning for prioritized diseases needs to be in place. Active surveillance is crucial for early detection of exotic diseases to address risks due to the long border with countries like Bangladesh from which there is an active movement of live aquatic animals and the import of items like ornamental fishes, which do not go through a full-fledged quarantine. In the likelihood of identification of an exotic disease, the emergency response measures would need the coordination of central and stage agencies and implementation of pre-decided course of actions. An exposure assessment under the following four stages: emergence of a pathogen, establishment in a farmed population, establishment at the larger scale and economic impact of disease - can help identify the risks and likely impacts. Such an assessment would help plan steps required to contain further spread. In the event of establishment of exotic disease, eradication is unlikely to be practicable under the present aquaculture scenario. So, measures to control and contain further spread of the disease would be required. An analysis of the controlled introduction of Litopenaeus vannamei in India and the response to the suspected presence of Acute Hepatopancreatic Necrosis Disease revealed strengths and weaknesses. Based on analysis the identified measures are: institutional strengthening, modification of regulatory processes, increased role of private sector, communication with stakeholders and the inter-agency coordination. To prevent further spread and minimize impact of endemic disease, focussed attention on diseases of concern based on economic criteria is critical. Further, disease epidemiology investigations should help develop management measures for controlling their spread. Paying attention to seed quality and focussing on hatcheries would help a great deal in cutting down the spread of diseases. Besides, risk of spread of disease due to use of wild collected live feed required attention. A system of testing broodstock and seed needs to be in place. Farm level biosecurity measures and capacity building of farmers would contain the spread of these diseases. Public awareness campaign to encourage cooperation from industry and the community is very much required. In light of the developments in

India, steps to strengthen the policy framework for preventing spread of diseases in the country are identified.

Modulation of Shrimp Immune System for Disease Management

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The sustainable intensification of crustacean aquaculture in Asia, dominated by the farming of penaeid shrimp species, continues to be beset by existing and emerging viral, bacterial and protozoan diseases for which there are no effective cures. For example: significant economic global impacts are caused by viruses (e.g. White Spot Syndrome Virus (WSSV), Infectious Myonecrosis Virus IMNV), Gram-negative bacteria (e.g. *Vibrio harveyi*, *Vibrio parahaemolyticus*) and protozoa (e.g. various Microsporidia, causing EHP or cotton tail disease).

Whilst the past decade has witnessed very significant advances in our understanding of the interactions between crustaceans and micro-organisms, we are still a number of years away from the identification of disease intervention strategies that are proven at farm scale. In the interim we must consider alternative mechanisms with which to modulate the shrimp immune system for disease management.

Moreover, it can be argued that to focus treatments on single pathogens, one at a time, is a limited approach that is not yet applicable to typical aquaculture systems seen throughout Asia (essentially pond culture). More sustainable solutions could be achieved by identifying the optimal environmental conditions that would minimise the disease outbreaks rather than attempting to eradicate the pathogen from each host.

Many diseases exist in wild populations but only become problematic when crop species are held at high densities and under stressful conditions. These conditions are often associated with aquaculture. Indeed, there is an environmental component to the aetiology of many crustacean diseases. For example, previously farmers in southern India have been able to maintain a good harvest of shrimp in spite of apparent signs of white spot syndrome virus (WSSV) infection, and it is known that WSSV infection outcome varies as a function of temperature acting on both the host and the virus.

This presentation will provide an overview of the latest insights of the crustacean immune mechanism and how it can be affected environmental manipulation, in the context of managing disease. From first principles, and using published examples of the regulation of infection outcomes in a wide variety of crustaceans, a case will be made for managing the pond environment to promote the health of the cultured crop. Where appropriate, the requirement to adopt a precautionary approach to implementing scientific research at farm scale will be emphasised.

Dr Chris Hauton *FRSB is a crustacean immunologist at the University of Southampton, UK. In 2008 he worked with the Malaysian ABT, providing training in 'Shrimp Health and Immunobiology' for fishery officers and shrimp farmers and in 2011 he was an invited speaker at an OECD workshop on 'Diseases of Beneficial Invertebrates' in Canada. He has worked extensively to quantify molecular immune mechanisms in the European lobster Homarus gammarus and used these data to reconsider the validity of immune priming strategies within the aquaculture industry. With Dr Valerie Smith, he has identified crustin antimicrobial peptides in H. gammarus and proposed a classification scheme for the crustins within the wider Decapoda. Previously he has argued for minimum reporting guidelines (MISA) for reporting of meta data in immune performance and infection studies within aquatic invertebrates. He is currently part of a team exploring the sustainable intensification of crustacean and fish aquaculture in India and Bangladesh in a project entitled: 'Poverty alleviation through prevention and future control of the two major socioeconomically-important diseases in Asian aquaculture,' (https://preventcontrol.org/) that is funded by the Indian DBT, UK BBSRC, and UKAid through the Newton Bhaba Global Research Partnership in Aquaculture.*

Invasive oomycetes are a serious threat to our natural and man-made aquatic ecosystems

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Oomycetes are fungal-like organisms that are classified as Chromalveolates and phylogenetically grouped with diatoms and brown algae. They are among the most important groups of disease-causing organisms in both agriculture and aquaculture and thus represent a huge threat for global food security.

Some aquatic species can cause serious environmental disasters, wiping out our native aquatic animals, for example European crayfish and also several amphibians have been severely affected or have become extinct. One particular pathogen, *Aphanomyces invadans,* is now approaching European waters and represents a devastating pathogen. It is a tremendous problem in countries where it has already arrived and has established (*e.g*. Bangladesh, India and parts of Africa). There are also other aquatic oomycete pathogens known including *Haliphthoros, Halioticida, Lagenidium, Atkinsiella* spp. that infect marine and brackish animals including lobsters, langoustines, abalone and prawns. *Saprolegnia parasitica* is one of the most destructive fish pathogens and is found in most fresh water environments around the world. Remarkably, very little is actually known about the biology of these aquatic pathogens. However, what we do know is that they are: 1) potential invaders of marine & fresh water habitats, 2) they can cause serious economic or environmental damage and most importantly, 3) they are all uncontrollable at present. An overview of these various animal pathogenic oomycetes is presented.

Furthermore, our current knowledge about the cellular and molecular infection strategies of *S. parasitica* and *A. invadans* is being discussed. In order to successfully infect their hosts these parasites rely on their effector repertoires. Effectors are secreted proteins of the pathogen that can alter the host and which advance the infection process. A large number of effectors is located at the interface between pathogen and its host and fulfil a function on the outside of the host cell. Such effectors are often classified as extracellular effectors. While several other oomycete effectors are able to translocate into their host cells where they can, for example, interfere with defence responses of the host. These effectors are commonly referred to as intracellular effectors. The mechanism of translocation or delivery of the intracellular effectors is under intense investigation and several routes of entry have been proposed. Here we will discuss our recent advances in understanding secretion, delivery and functions of effectors from animal pathogenic oomycetes.

Pathogen Trapping by Defence Cells of Fish and Shellfish

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The controlled release of chromatin from the nuclei of neutrophils or other inflammatory cells (a process now known as ETosis) has, over the last fifteen years, become recognised as an important microbial trapping strategy in higher vertebrates. Paradoxically, however, it is becoming clear that, in addition to contributing to host protection, this defence process may, in mammals, also be associated with certain pathological states. These include venous thrombosis, cancer metastasis and some autoimmune diseases caused by production of auto-antibodies against immunogenic complexes of self-DNA. This raises questions as to whether ETosis occurs in lower taxa, including fish and shellfish, and whether or not it has negative effects for these animals. Analyses of crab, mussel, oyster and shrimp haemocytes, as well as earthworm coelomocytes, and mesogleal cells from sea anemones, confirm that invertebrate phagocytes can undergo ETotic-like expulsion of chromatin *in vitro.* The response in crabs is very similar to that of neutrophils in higher vertebrates as it involves NADPH oxidase, actin filamentation, and histone citrullination, which together lead to the ejection of the nuclear material to the extracellular environment where it forms a cloud-like mesh that entraps bacteria in the vicinity of the dying cell. Related *in vivo* experiments, also in crab, further show that the chromatin meshes occur within the haemolymph where, crucially, they act as the scaffold upon which intact haemocytes assemble during encapsulation reactions. Importantly, because the extruded DNA nets become studded with histones and other antimicrobial proteins, the ensnared micro-organisms are brought into close contact with agents that kill them. Clearly the deployment of chromatin is an ancient defence weapon, as mesogleal cells from an acoelomate exhibit ETotic behaviour. It appears to benefit animals without complex tubular circulatory systems or highly specific antibodies and, so far, there been no reports that ETotic reactions have adverse consequences on the host. The situation for fish, on the other hand, is less clear as these animals possess fully tubular vascular systems and have the ability to produce three classes of immunoglobulin antibodies, yet still retain strong reliance on the innate responses of the immune system for host protection. Evidence from a variety of *in vitro* studies has established that fish head kidney leucocytes do indeed cast chromatin nets after stimulation by bacteria, lipopolysaccharide or zymosan as well as calcium ionophores, although with highly enriched neutrophil populations there may be a small proportion of ETotic cells in the

control cultures, perhaps as a result of the enrichment procedures of the head kidney samples prior to culture. In salmonids, at least, the process differs somewhat from that in mammals as it does not seem to rely on protein kinase c activation and is not blocked by NADPH oxidase inhibition. That the chromatin fibres disgorged from the highly enriched neutrophil populations become decorated with histones and neutrophil elastase, like their mammalian counterparts, supports the view that functionally the process is genuinely ETotic. In salmonids, too, enriched cultures of head kidney macrophages have also been found to be capable of an ETotic response as, surprisingly, so do a small number of erythrocytes, (which teleosts are nucleated) in most teleosts. By contrast, peripheral blood lymphocytes from salmon seem not to display the phenomenon despite published reports that some fish B cells are able to phagocytose small particles. Preliminary findings on salmon are also revealing the presence of externalised chromatin material in wax sections of tissues taken from naturally infected fish, indicating that ETosis occurs *in vivo* under disease conditions. Interestingly, some fish pathogen strains, such as *Aeromonas salmonicida or Listonella (Vibrio) anguillarum,* express DNAses, which, theoretically, could dismantle the net structure and allow the pathogen to escape entrapment and killing. Further investigation of this is currently underway but from the data obtained so far, it is likely that the interaction of pathogens with ETotically-derived extracellular chromatin is a significant factor in determining fish and shellfish health. However, as yet, it remains unknown if the process of externalisation of nuclear material through ETosis is a double-edged sword for fish in a parallel way to that for mammals.

Molecular Insights in Host-Parasite Interaction and Implications for Developing Novel Control Strategies

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Worldwide, parasitic diseases of aquatic animals impose considerable constraints to the expansion and management of aquaculture, thus attempts to control diseases have become a main concern in many fish-producing areas. How parasites recognize the suitable hosts or cells, yet at the same time evade recognition and elimination by the host immune system. The mechanisms of such recognition and how to avoid recognition are ruled largely by host-parasite surface membrane interactions at the cellular and molecular level. Unique molecules produced by unusual pathways of these parasites are involved and play important roles in their survival in the host.

Small-interfering RNA (siRNA) has emerged as a potential tool to investigate gene function and host pathogen interactions, and also to control infection and growth of many pathogens that lead to severe ecological and economical distress in aquaculture industries. We applied the siRNA approach to prove the efficacy of this strategy in controlling two important parasitic diseases of fish.

Myxobolus cerebralis, the causative agent of whirling disease, which alternates between two hosts; a salmonid fish and an invertebrate oligochaete, *Tubifex tubifex*. siRNA targeting MyxSP-1 (a serine protease implicated in whirling disease pathogenesis), induced RNAi in vivo which abrogated the *M. cerebralis* life cycle in the oligochaete host providing a proof-of-concept interference approach in salmonid whirling disease. Additionally, siRNAs targeting the intracellular parasite *Heterosporis saurida* both ADP/ATP transporter and Methionin aminpeptidase genes could specifically and differentially silence transcripts encoding these proteins and inhibited parasite replication in vitro. Proliferative kidney disease (PKD) is a myxozoan parasitic disease that significantly affects both farmed and wild salmonid fish in Europe and North America. PKD is caused by the myxozoan *Tetracapsuloides bryosalmonae*, the life cycle of which is alternated between invertebrate freshwater bryozoan and vertebrate salmonid fish hosts. The infected brown trout transmit the parasite to bryozoan colonies but not rainbow trout. We identified remarkable kidney swollen index, parasite numbers and parasite quantity in infected-brown trout and rainbow trout. We have identified differentially expressed transcripts in infected kidney of brown trout and rainbow trout by using suppressive subtractive hybridization and confirmed their transcriptional levels in the infected kidneys. Our study suggests that differential expression of host anti-inflammatory, humoral immune and endocytic pathway responses, cell proliferation, and cell growth processes do not inhibit the development of intra-luminal sporogonic stages of the European strain of *T. bryosalmonae* in brown trout but may suppress it in rainbow trout. The infected brown trout can release viable spores into the water bodies for more than two years.

Epizootic Ulcerative Syndrome (EUS) is one of the most destructive fish diseases caused by the aquatic oomycete, *Aphanomyces invadans*. Proteases produced by many microorganisms, including oomycetes, are crucial for their growth and development and some also play critical role in disease manifestation .The mechanism by which the fungus induces the disease is still unclear. Dominant protein bands of extracellular products (ECP) of *A. invadans* were analyzed by mass spectrometry and their proteolytic activity was investigated against immunoglobulins of both giant gourami (*Osphronemus gorami*) and rainbow trout (*Oncorhynchus mykiss*). The mass spectrometer analysis identified proteases, mainly serine proteases in ECPs. The secreted proteases degraded immunoglobulins of both fish species while the protease inhibitors, mainly serine protease inhibitors inhibited the protease activity in the ECPs. We plan to edit *A. invadans* genome to knock out these proteases and affect the virulence of the oomycete. To test this notion, we aim to edit the identified protease genes using CRISPR/Cas9 system.

Understanding the mechanisms and pathways underlying fish parasite interaction is essential to develop an effective strategy for disease management in aquaculture.

Emerging Aquatic Animal Diseases in the Asia-Pacific Region

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The Asia-Pacific region produces around 90% of the world aquaculture production. Aquaculture in the region is a highly significant food production sector that provides many livelihood opportunities, and contributes to food security, nutrition and poverty alleviation. Despite the significant developments in the aquaculture sector, the industry faces many challenges and problems that affect sustainable production. Disease outbreaks, specifically transboundary aquatic animal diseases, are one of the serious causes of production losses in many aquaculture operations around the region. Most are mainly a result of transboundary movement (trade) of live aquatic animals. Several transboundary aquatic animal diseases have swept the region over the past 25 years which have caused massive economic and social losses. These include spread and outbreaks of epizootic ulcerative syndrome (EUS) in freshwater fish, viral nervous necrosis (VNN) in marine fish, viral haemorrhagic septicaemia (VHS) in marine and freshwater fish, and several viral diseases in shrimps (e.g. WSD, IHHNV). Recently, outbreaks of Acute hepatopancreatic necrosis disease (AHPND), popularly known as early mortality syndrome (EMS), among cultured shrimps were reported in the region including China and Vietnam (2010), Malaysia (2011), Thailand (2012), and the Philippines (2014). The spread of these transboundary diseases clearly demonstrates the vulnerability of the aquaculture industry to disease emergence where impacts have been aggravated by the lack of effective preparedness and response when diseases emerge.

The risk is still very high that these important aquatic animal diseases will spread, as transboundary movement of live aquatic animals within and outside the region is inevitable. At present, emerging diseases are now affecting production of major cultured species in some aquaculture producing countries. These diseases pose significant effect in major cultured species in terms of production and economics, once they start to spread from one country to another. Some of these diseases have already been reported to be widespread, but significant outbreaks are not yet reported or might not have been brought to the attention of the responsible Competent Authorities. This paper will present status of some emerging disease problems including Hepatopancreatic microsporidiosis caused by *Enterocytozoon hepatopenaei* (HPM-

EHP) and Viral covert mortality disease (VCMD) in shrimps, and Tilapia-lake virus and Carp edema virus in finfish.

By and large, outbreaks of damaging aquatic animal diseases are likely to continue and the potential consequences are likely to increase with the expansion (intensification) of aquaculture systems, and introduction of new species for culture. Consequently, the risks associated with emerging and transboundary diseases are shared – shared water bodies and epidemiological links through trade (especially live movement) – thus, collaborative approach (in dealing with these diseases) is therefore warranted and necessary.

Health Management and Biosecurity in Shrimp Aquaculture in India- A review

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According to Chamberlain, 2011, 'shrimp aquaculture is a young and dynamic business with a history of rapid change in response to technological advances'. The Indian shrimp aquaculture scenario also truly reflects this. Similar to terrestrial animal production system, shrimp aquaculture had evolved from the traditional farming of wild stocks at low density in more or less natural settings to an intensive culture based on domesticated stocks in a more controlled environment. Indian shrimp farming has witnessed this transformation over the last three decades. Shrimp farming using post-larvae collected from tidal waters had been in practice in the states of Kerala and West Bengal for centuries and is being continued as a low input low production system. However, the development of hatchery technology for the mass-scale seed production of tiger shrimp, *Penaeus monodon* along with the involvement of farmers and timely intervention by agencies like Marine Products Export Development Authority (MPEDA) and ICAR fisheries research institutions paved the way for the development of scientific shrimp farming in India in the 1980s. From 1980s until 2009, the shrimp industry was relying solely on wild-caught broodstock of *P. monodon* for seed production. Despite this constraint, the farming sector witnessed spectacular growth during this period.

This period was characterized by unregulated expansion and intensification and has been marked by two significant events which had far reaching implications on shrimp aquaculture in the country. The first one was the unprecedented disease outbreak which devastated the shrimp farming industry. The first major disease outbreak in commercial shrimp farms was reported during 1993-1994 from the farms located around the Kandleru creek in Andhra Pradesh leading to large-scale mortality. The causative agent was initially reported as systemic ectodermal and mesodermal baculovirus (SEMBV) and later, identified as white spot syndrome virus (WSSV). Subsequently, the disease spread to all the shrimp farming areas. WSSV continued to inflict heavy damage to the shrimp farming and the sector took almost 4–5 years to revive from this situation through precautionary approach and the adoption of good management practices. The implementation of science-based health management procedures has been a turning point in Indian aquaculture, as historically, diseases and pathogens did not get much attention until this period. The rethinking of health management and biosecurity practices happened elsewhere in the world too because of the global epizootic caused by WSSV.

The second significant event was related to the environmental regulatory aspects of shrimp culture in which the Supreme Court of India on 11th December 1996 handed out crucial verdict with major implications for the shrimp aquaculture sector. Further, following the directives of the apex court, the Aquaculture Authority of India was established in 1997 under the Environmental Protection Act, 1986 and subsequently, the Govt. of India, through an Act of the Parliament established the Coastal Aquaculture Authority (CAA) in 2005 for regulating the aquaculture activities.

Well before the WSSV epizootic occurred in shrimp aquaculture, Epizootic ulcerative syndrome (EUS) was reported during 1980s in finfishes and, this is the first transboundary fish disease recorded from India. Of the 26 OIE-listed pathogens, 3 crustacean diseases and one each of finfish and molluscan diseases have been reported so far from Indian aquaculture. Although along with other pathogens, viruses such as monodon baculovirus (MBV), hepatopancreatic parvo virus (HPV) and Leam Singh virus (LSNV) have been reported from farmed shrimp, the economic impact these viruses cause has not been comparable to WSSV. To combat the increasing incidents of diseases, progressive changes in aquatic animal health management have been taking place and a number of strategies/approaches and efforts were made in the field. These include the preparation of national strategic plan and guidelines for aquatic exotics and quarantine, development of sensitive diagnostics and capacity building in disease diagnosis, harmonization of diagnostic tests and accreditation of PCR laboratories, developing, disseminating and implementing best management practices (BMPs) through cluster-level approach. The efforts were also directed towards the development of therapeutics and prophylactics including vaccines.

The most significant transformation that happened in Indian shrimp farming, post-WSSV, is the introduction of the non-native species, whiteleg shrimp, *P. vannamei* replacing the dominant native shrimp *P. monodon* during 2009–10. The introduction had been preceded by the approval of National Committee on Introduction of Exotic Species, risk analysis assessment and formulation of specific guidelines for commercial-scale farming of SPF *P. vannamei* and the review of the recommendations by Coastal Aquaculture Authority (CAA). This was followed up with the stringent regulatory measures such as mandatory registration of shrimp farms, stringent quarantine of imported broodstock and biosecurity measures at farms and hatcheries. These efforts resulted in the rejuvenation of shrimp farming in the country. After the introduction of the species, export production has grown from 1731 tonnes in 2009 to 35, 3413.1 tonnes in 2014–15. However, lately, as witnessed earlier in the case of native tiger shrimp, diseases of infectious aetiology as well as morbidity associated with obscure aetiology and poor management, use of pond-reared broodstock for seed production etc., have caused serious concerns to *P. vannamei* farming. Despite farming post-larvae derived from WSSV-free broodstock, the most important production constraint remains to be WSSV. However, significant production losses due to multiple disease conditions such as running mortality syndrome (RMS), zoea syndrome, retarded/slow/stunted growth, white faeces/white gut syndrome etc., have frequently been reported in farmed *P. vannamei*.

Indian aquaculture sector has been confronted with many issues such as lack of surveillance data on diseases of aquatic animals; lack of data on socio-economic impact of diseases; lack of coordinated, harmonized and synergistic research efforts; limited information on chemicals and biologicals used in aquaculture etc.. However, in the past few years, these issues have been addressed through focused efforts of various stakeholders. These include an expert consultation on transboundary diseases at a regional level in 2012 followed by the introduction of Agriculture Biosecurity Bill, 2013 in the parliament and the proposal to establish the National Biosecurity Authority. Significantly, National Surveillance Programme for Aquatic Animal Diseases was initiated in 2013. The surveillance programme resulted in the creation of a database on aquatic animal diseases/pathogens and identification of some of the pathogens which have not been recorded before. Recently, ICAR has made significant financial investment to implement the All India Network Project on Fish Health and ICAR-Consortia Research Platform (CRP) on Vaccines and Diagnostics. In conclusion, health management and biosecurity have become the most significant component of aquaculture production system and this represents a history of rapid change in response to the ever-growing demands of the sector supported by technological advances.

Current Status of Molluscan Diseases in India

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India has a rich bivalve fauna consisting of clams, mussels, edible oysters and pearl oysters. Besides serving as a food source, they provide valuable foreign exchange earnings, employment and income to the coastal fisher folk of the country. One of the greatest advantages of culturing bivalves, is that they do not require any external feed inputs as they feed on freely available natural plankton in the water, thereby minimizing the major input costs as well as the environmental impacts. They also remove the suspended organic matter in water through continuous filtration helping to reduce eutrophication of estuaries, bays and near-shore waters. Green mussel, *Perna viridis*, Indian backwater oyster, *Crassostrea madrasensis* and the pearl oyster, *Pinctada fucata* are the important cultured species. During the past decades, commercial mussel culture activity in the estuaries along the south west coast of India, especially in north Kerala has picked up in a big way with the production reaching 18,432 tonnes in 2009. But outbreak of diseases has caused a sharp decline in the production during the past few years.

Diseases are the most important limiting factors for any aquaculture venture and the risks increase with the intensification of culture activities. Over the past few decades, world molluscan production has been adversely affected by a number of diseases. International Disease Commission (OIE) has listed the diseases caused by *Bonamia ostreae, B. exitiosa, Marteilia refringens, Perkinsus marinus, P. olseni, Xenohaliotis californiensis* and Abalone virus as the most important diseases in molluscs. Indian bivalve culture has also started feeling the heat, with OIE listed diseases like *Perkinsus olseni* causing mass mortalities in green mussel farming.

Bivalve pathology in India is in nascent stage and the available information on pathogens is very limited. Losses due to diseases were insignificant when the production was low, but the rapid expansion of bivalve aquaculture is changing the way things are viewed. The first report of an OIE listed pathogen *P. olseni* from India was that infecting the pearl oyster, *P. fucata* in 2010. Another strain of *Perkinsus, P. beihaiensis* was reported from *C. madrasensis* in 2012. Since 2013, under the National Surveillance Programme for Aquatic Animal Diseases (NSPAAD), regular monitoring of bivalves for OIE listed pathogens was initiated. Mussel/oyster farms in 6 districts of Kerala were monitored. Wild bivalves along the East and West coasts of India as well as the Andamans and Lakshadweep islands were also screened. A total of 5226 samples from 25 species of bivalves were analysed. The most prominent pathogen observed along the west coast was *P. olseni.* Stray cases of *P. olseni* were observed from the east coast. *P. olseni* exhibit a very wide host range and has been reported from twelve bivalve species in India. High prevalence of *P. olseni* is observed in farmed and wild green mussel, *Perna viridis* and the clam, *Paphia malabarica* along the Kerala and Goa coasts. Infections with *P. beihaiensis* was observed in *S. cucullata, C. madrasensis* and *P. malabarica*, while *P. malabarica* & *S. cucullata* harboured both *P. olseni & P. beihaiensis.*

Prevalence of *P. olseni* has been showing an increasing trend over the past two years and mass mortalities in farming systems were reported during 2015-16. Movement of infected bivalves/seeds for trade/culture/consumption can aid the rapid spread of the disease. Since the past few years there has been a perceptible change in the climatic pattern manifested by an increase in temperature. Higher temperature and salinities are always stressful to mussels and such conditions are known to increase the pathogenicity and associated mortalities caused by *P. olseni* all over the world. Stress induced by the drastic changes in climate weakens the hosts giving an upper hand to the pathogens, leading to heavy mortalities in them.

Infection with *Bonamia* spp. was observed (through molecular diagnosis) in *C. madrasensis* and *S. cucullata* from Gujarat and in *S. cucullata* from Lakshadweep islands (unpublished).

Infection with Osterid Herpes Virus has been reported from bivalves world over, but so far it is not reported from India and all the screened samples were negative. . The only information on bacterial pathogens is that of mass mortalities of *P. fucata* larvae in hatcheries in 2009, caused by *Vibrio* sp. Infections with Rickettsia-like organisms (RLOs) have been reported from *C. madrasensis* along the west coast of India.

Information on other pathogens/parasites of bivalves in India is limited to reports of Copepod parasites (*Mytilicola* sp.), polychaete worm (*Polydora* sp.), trematode larvae (*Bucephalopsis haimeanus*) in *C. madrasensis* and *Tylocephalum* sp. in *Pinctada fucata.* Infections with *Anicistrocoma* like ciliates and *Steigotricha* sp. infecting the digestive tubules, *Ricketsia* like organisms, *Nematopsis* sp., *Trichodina* sp., turbellarians, nematodes, trematode metacercaria, pea crabs (*Pinnotherus* sp.), boring sponge (*Cliona* spp.) etc. have also been reported.

As the bivalve farming in the country is in the initial phase of expansion, we have an opportunity to put a scientific health management strategy in place. In this context, it is essential to specifically identify and document the various pathogens/parasites present in the mussel populations, both wild and farmed so that strategies can be drawn to prevent/control their spread. A proactive strategy for prevention of diseases is the only viable alternative in aquaculture disease management for which the basic pathogen profile of the farmed species is very important.

Aquatic Animal Epidemiology: A Cross-Learning from Terrestrial Animal Epidemiology

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The advances in information technology provides adequate computing techniques to develop a national livestock disease information system which is the prime need of the day. ICAR-National Institute of Veterinary Epidemiology and Disease Informatics has developed an innovative offline india.admasEpitrak and online NADRES epidemiology software which is a dynamic and interactive livestock disease relational database supported by Geographic Information System (GIS). This software addresses the needs of data collection, retrieval, analysis and critical reporting of disease events as and when they occur for developing appropriate control measures.

ICAR-NIVEDI provides sampling plan for all the centers of AICRP for conducting sero-epidemiology of livestock diseases. Sampling plan is structured with in-built village level livestock population and advanced statistical & analytical methods. The environmental and remote sensing variables generated at village level to develop disease –climate relationship models and risk maps. Risk maps are more useful for optimum utilization of resources and control of livestock diseases, these risk maps provides simplified solutions and amplified opportunities. A multivariate logistic regression is being used to forecast the probable disease occurrence in two months advance, monthly forecasting report is submitted to department of Animal husbandry Dairying and Fisheries (DADF), Government of India for dissemination and alerting the state government officials to take appropriate preventive measures. Forecasting results are evaluated for accuracy ranging from 80-90% with low false negative rates. Two months advance disease alerts are issued for 13 livestock diseases at district level every month. Further work is in progress to develop the disease alert system at block levels.

Risk factors analysis is performed to identify the significant risk factors associated with disease occurrence. Risk factors analysis along with risk mapping supports to develop risk based surveillance system (RBS) which is more cost-effective compared to traditional surveillance system.

To conclude the existing animal disease forewarning and forecasting systems in the country may be replicated in fisheries with necessary modifications for supporting the policies and taking appropriate actions during aquatic animal disease emergencies for control and prevention. Presently ICAR-NIVEDI is providing the support to develop database on national surveillance program for aquatic animal diseases (NSPAAD), which is reliable and robust informatics and predictions of aquatic animal diseases in India. The only online software (http://49.50.73.242/nspaad_live/) of aquatic animal disease database and epidemiological analysis presently available in India.

Current Status of Freshwater Fish and Shellfish Diseases in India

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Aquaculture, particularly freshwater aquaculture is now integral to the economies of many countries including India. It is the second largest producer of fish and also second largest producer of freshwater fish in the world. The total fish production in the country during 2015-16 is about 10.79 million metric tonne (MMT). The inland fisheries sector contributes about two-third of the total production and freshwater aquaculture sector alone contributes about 85% of the total inland production. The three Indian major carps , namely catla, rohu and mrigal contribute to bulk of production with production over 3.25 million tonnes. Exotic carps viz., grass carp, silver carp and common carp are the next important species in culture systems. Besides, catfish, murrels and freshwater prawns are also being widely cultured. In last few years, the exotic catfish *Pangasianodon hypophthalamus* and pacu, *Piaractus brachypomus* culture are also increasing. As there is limited scope for horizontal expansion, the current trend in aquaculture development is towards increased intensification. But, diseases and epizootics are considered to be major bottlenecks for increasing production. It is estimated that diseases account for 10-15% towards the production cost. In one of the pilot studies, loss due to an ectoparasitic disease argulosis has been estimated to be to the tune of Rs 29,524.40 (US$615) per hectare per year in carp culture. Further, losses due to infection with *Aphanomyces invadans* has has been estimated to be in the range of Rs.10000-26000/-hectare. In freshwater prawn industry white tell disease is considered to be mainly responsible for its crippling down causing high mortalities and huge economic losses (Hameed *et al.,* 2004).

Viral diseases

More than 125 different viruses have been identified in fish around the globe and new viruses are being discovered at an alarming rate. However, there are a few reports of viral diseases affecting finfish from the country. The viral diseases that have been reported from the country include, Cyprinid herpesvirus-2, koi ranavirus (KIRV), carp edema virus (CEV), megalocytivirus from ornamental fish, and viral encephalopathy and retinopathy (VER). Goldfish haematopoietic necrosis herpes virus along with multi-drug resistant *Aeromonas hydrophila* were identified as the cause of large-scale mortality in goldfish from Hooghly district, West Bengal. Thereafter, CyHV-2 infection has been found to be widespread in diseased and apparently health goldfish samples. Further, KIRV has been reported to associated with mass mortality of koi, *Cyprinus carpio* in a farm in South India. In addition, koi sleepy disease caused by CEV has been diagnosed from a disease outbreak in adult koi, *Cyprinus carpio koi* from a fish farm in Kerala (Swaminathan et al., 2016). Subsequent screening of koi in Tamil Nadu, Kerala and Odisha revealed that the pathogen is quite widespread. Although marine or brackishwater finfish culture has not made a breakthrough in India, a major viral pathogen, betanodavirus (VER) is repeatedly being reported to be involved in mass mortality in seabass farming, even when cultured in freshwater. On the other hand, most of the OIE reportable diseases of viral origin in fish are yet to reported or recorded from Indian aquaculture systems even with the current active targeted surveillance system.

Bacterial diseases

Bacterial diseases like motile aeromonads septicaemia, edwardsiellosis, *Pseudomonas* septicaemia, flexibacteriosis, bacterial gill disease, streptococcal septicaemia, mycobacteriosis, columanaris disease and enteric septicaemia are often being reported in various culture systems of India. However, motile aeromonad septicaemia is considered to be the most common and troublesome amongst bacterial diseases of fish. The disease is caused by the gram-negative bacteria *Aeromonas hydrophila*, *A. sobria*, *A. caviae* and few other aeromonads. Many freshwater fishes such as Indian major carps, murrels, catfish, goldfish, tilapia, *Puntius* sp., are affected by this disease. Another septicaemic disease called edwardsiellosis caused by the gram-negative bacterium *Edwardsiella tarda*, has been reported to affect most of the freshwater fishes and all age groups of fish are susceptible to this disease. In addition, columnaris disease caused by *Flavobacterium columnare* is also an important disease of farmed fishes. Although not from major outbreaks, few other bacterial pathogens viz., *A. sobria, A. veronii, A. liquifaciencs*, *Proteus mirabilis, Klebseilla pneumoniae, Plesiomonas shigelloides* and *Acinetobacter baumannii* have been frequently being isolated from diseased fish.

Parasitic diseases

Parasitic diseases pose a major problem to the aquaculture sector and are responsible for significant economic losses. Argulosis caused by crustacean ectoparasites of the genus *Argulus* is one of the most important parasitic diseases of freshwater aquaculture particularly in cultured carp species and responsible for causing heavy economic losses. Infestation with *Lernaea* spp. (anchor worm) is also a common problem in cyprinids, including Indian major carps, koi carp, common carp, goldfish etc. In addition, some of the important protozoan diseases which have been reported to cause heavy infections in freshwater fishes include pathogenic trichonids (*Chilodonella, Trichodina, Tripartiella* and *Trichodinella*) and *Ichthyophthirius multifiliis* responsible for causing Ichthyophthiriasis or "white spot' disease. Further, some of the important monogenetic species *viz*. *Gyrodactylus* and *Dactylogyrus*, causing gyrodactylosis and dactylogyrosis, respectively are reported to be responsible for large number of outbreaks in freshwater aquaculture farms in India.

Fungal diseases

Infection with *Aphanomyces invadans* is considered to be one of the most destructive diseases affecting fresh and brackishwater farmed and wild fishes. After three decades of its first occurrence in the country, till today, the disease is considered to be a major problem in North and Northeast India during winter months. The Indian major carps, the major cultured species of the Indian sub-continent are highly susceptible. It is the only OIE listed finfish disease reported from the country. Another important disease, saprolegniasis is caused by a water mould *Saprolegnia parasitica*. The disease affects most of the freshwater fish species including carps, catfishes, tilapia, trouts etc. In addition, *Achlya* sp., *Dictyuchus*, and *Pythium* have been isolated from Indian major and minor carps, exotic carps like grass carp, common carp, banded gourami, climbing perch, snakehead and catfish.

Conclusion

With food security being a primary concern for the country, the aquaculture sector has the potential to play an important role for providing quality animal protein for the growing population besides providing livelihood for people. To meet the future demands, sustainable growth of the aquaculture sector is crucial. As intensification and diversification will continue to increase to meet the demand, these will led to the emergence and spread of new diseases. Early detection is considered to be a key to the control of diseases. This highlights the need of a strong surveillance and monitoring system in the country.

Principles and Applications of Randomized Control Trials of Aquaculture Treatments and Vaccines

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Disease outbreaks in aquaculture often affects a large number of animals due to the nature of disease transmission in aquatic environments and husbandry practices that increase exposure to multiple groups. Delivery of treatments are usually limited to oral (in feed) or to topical bath treatments. Injection is uncommon except perhaps for some vaccinations or in some broodstock situations. Treatment options are complicated by the fact that sick individuals who most require treatment are usually not eating medicated feed and bath treatments using reduced water volume (to reduce the overall amount of topical medication required) can compromise health due to handling or crowding. Ultimately, with many variables involved, field treatments are economic costs that, even when optimized, may not deliver the benefits suggested by experimental results in a laboratory.

Disease prevention is a key strategy for lowering the impact of infections and reducing the productivity consequences of infectious agents. Vaccines have been developed for many pathogens but their benefits are rarely assessed in the field using rigorous testing methods. Decisions regarding the use of treatments and vaccines require rigorous evidence to assess their benefits and possible adverse reactions. Choosing a disease control option cannot rely on expert opinion of what should work, it should be based on evidence that it does work. The best quality evidence for control decisions is derived from appropriately designed randomized control trials (RCT).

This presentation will address critical components of RCTs, many of which require novel approaches to implement in aquaculture situations. Selection criteria for animals eligible to be included in the trial are critical to the external validity (i.e. the capacity to generalize to other populations) of the conclusions. Identification of the units to which the treatment allocation occurs can be confusing when applied to aquaculture conditions. The pond or tank of fish may be the best option but this group will need to be maintained throughout the data collection period and this unit must be incorporated into the case definition and analysis. Internal validity of the trial (i.e. a measure of the treatment effect in the study population) requires unbiased allocation of treatment units to treatment and control groups, and blinding of individuals to reduce differential management of comparison groups or influencing the classification of cases. Although randomized control trials provide the strongest evidence for quantifying the effect of the control measure, their implementation in aquaculture is sufficiently difficult that they are frequently circumvented or ignored. Progress in disease management demands appropriate RCT because ongoing treatment decisions, particularly ones that affect a large proportion of farmers, should not be based on opinion or conjecture in the absence of critically assessed evidence.

Abstracts

ISAAHE-001

Incidence of Different Diseases in Freshwater Aquaculture Systems through Surveillance in Two Important Aquaculture States of India (Andhra Pradesh and Odisha)

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Diseases are the major constraints for the development of the aquaculture industry in a sustainable manner. To know the presence of pathogens/diseases in freshwater aquaculture farms of two of the important aquacultured states of India, Andhra Pradesh and Odisha, both passive and active targeted (koi herpes virus and spring viraemia of carp virus) surveillance was undertaken during 2014-2017. Baseline information with GPS coordinates of 1271 farms were generated during the period. Under active surveillance, total 3,126 individual samples screened for spring viraemia of carp virus and Koi herpes virus were found to be negative by level III diagnosis. Further, 135 numbers of passive cases were examined using level II and level III diagnosis systems. From the passive samples, 63.70% were parasitic, 20.70% bacterial, 11.10% mixed bacterial and parasitic, 2.20% viral and 0.7% cases of miscellaneous disease conditions. Among the parasites, *Argulus* (30.6%), *Dactylogyrus* (22.44%), Myxosporideans (8.16%), *Ichthyophthirius* (5.10%), *Trichodina* (2.04%) and mixed parasitic infections (34.68%) were found. Among the bacterial pathogens, Aeromonads were found to be the dominant group of bacteria causing fish mortality (71.1%). Within aeromonads, *Aeromonas hydrophila* (62.50%), *A. sobria* (25%), *A. veronii* (9%), and *A. caviae* (3.1%) were recorded based on multiplex and 16S PCR followed by sequence analysis. During the period, three emerging viral pathogens also have been reported viz., Cyprinid herpesvirus-2, Carp edema virus and betanodavirus from goldfish, koi carp and wild seabass, respectively. Cyprinid herpesvirus-2 seems to be the first report of viral disease outbreak in ornamental freshwater farm from India. Some bacterial pathogens of zoonotic potential causing fish mortality also have been reported viz., *Plesiomonas shigelloides, Proteus mirabilis, Klebsiella pneumoniae,* and *Acinetobacter baumannii*. The antibiogram and integron assay for few of the bacterial pathogens were studied, and the nature of multidrug resistance was noticed.

ISAAHE-002

Surveillance of Freshwater Fish and Shellfish Diseases in Uttar Pradesh and Haryana

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The major emphasis of the ongoing surveillance programme has been to strengthen the farmer based surveillance in both the states. For achieving the same, awareness was created amongst 1363 fish farmers and 123 fisheries officers regarding important fish diseases and their symptoms. In addition, training was provided to 18 fisheries officers for strengthening their diagnostic capability. For active surveillance, 6 districts in Uttar Pradesh and 5 districts in Haryana were selected and baseline information of a total of 933 farms has been collected and entered in the database. During the reporting period, sampling was carried out from a total of 335 fish farms, and 2516 fish samples were collected and screened for SVCV and KHV. All the samples were found negative. However, screening of ornamental goldfish from different cities of Uttar Pradesh indicated that Cyprinid herpesvirus-2 infection was found to be quite widespread (64 out of 306). In addition, a total of 390 shrimps samples were collected from 12 farms of Haryana, a landlocked state where shrimp culture is picking up using ground saline water. These samples were found to be positive for *Enterocytozoon hepatopenaei,* whereas all the samples were negative for WSSV, IHHNV and HPV. During the reporting period a total of 46 disease outbreak cases were investigated which included 21 cases of infection with *Aphanomyces invadans*, 3 cases of *Aeromonas hydrophila* infection, one case of *Shewanella putrefaciens* in *Oreochromis niloticus,* one case of *Saprolegnia ferax,* one case of epitheliocystis, 15 cases due to water quality problems in fishes and 3 cases of hepatopancreatic microsporidiosis in shrimps. The present surveillance programme has been helping in increasing the awareness of the farmers about fish diseases and their management.

ISAAHE-003

Surveillance of Ornamental Fish Diseases

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Ornamental fish samples (n = 1025) were collected from Kerala and Tamil Nadu for aquatic animal disease surveillance during 2013-2017. In the active surveillance, the pooled samples were screened for important OIE pathogens viz., Koi Herpes Virus (KHV) and Spring Viraemia of Carp Virus (SVCV) and all samples were found negative. Emerging pathogen Cyprinid Herpesvirus 2 (CyHV-2) causing haematopoietic necrosis disease in goldfish was isolated, identified and confirmed from a disease outbreak in an ornamental fish farm at Hoogly district, West Bengal and the nucleotide sequence analysis revealed that the Indian strain of CyHV-2 appears to be different from China and Japan strains. Based on major capsid protein gene, a new set of nested primers was designed for detection of virus which could detect 0.01 pg of CyHV-2 DNA. CyHV-2 virions were purified using sucrose gradient ultra-centrifugation and confirmed by PCR and transmission electron microscopy. Another viral pathogen, carp edema virus (CEV) causing "Koi Sleepy Disease"(KSD) in koi was identified from ornamental fish farms in Kerala and Tamil Nadu. Sequence analysis of partial 4a gene of CEV suggested that Indian CEV is genetically distinct strain. Four cell lines of *Cyprinus carpio koi*, *Carassius auratus, Pterophyllum scalare* and *Astronatus ocillatus* were developed for virus isolation. Cutaneous fibroma with systematic mycobacteriosis and infiltrative lipoma in goldfish were confirmed by histopathology. Ornamental fish disease management consultation was given to 110 ornamental fish farmers and 80 antibiotic sensitivity tests were carried out to prescribe the correct antibacterial agent to treat the diseased fish.

ISAAHE-004

Surveillance of Fish and Shellfish Diseases in Selected Districts of Tamil Nadu, Andhra Pradesh, West Bengal and Pondicherry

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Disease surveillance is being carried out under the NFDB funded NSPAAD project to monitor the diseases of shrimp and freshwater fish in selected districts of Tamil Nadu since 2013. Water and animal samples have been collected on monthly basis. Various physic-chemical characteristics of pond water have been measured using standard procedures. Animal samples were analysed by PCR, RT-PCR, histopathology and bacteriological analyses. Baseline data including GPS coordinates were collected for 135 shrimp farms located at Tamil Nadu, Andhra Pradesh and West Bengal, and 87 freshwater fish farms in Tamil Nadu and Pondicherry. The occurrence WSSV infection was found to be the most prevalent infection for the past four years of study in our surveillance area. Next to WSSV infection, *Enterocytozoon hepatopenaei* (EHP) was found to be a serious problem in the culture system. Shrimp samples collected from different locations in Tamil Nadu and Andhra Pradesh were screened for EMS and our results helped the concerned authority to declare Indian shrimp culture system free from EMS. The occurrence of IMNV was reported for the first time in Indian shrimp culture system. Multiple infection caused by WSSV and EHP was reported in some shrimp farms. Supplied positive controls and fish cell lines to the NSPAAD project partners. Trained research scholars and scientific personnel in molecular diagnostics and fish cell lines. Disease awareness meeting was conducted on many occasions at different paces for the benefit of farmers and also to popularize the NSPAAD project activities among the shrimp/fish farmers.

ISAAHE-005

Surveillance of Fish And Shellfish Diseases in Nagapattinam, Pudukkottai and Thanjavur Districts of Tamil Nadu

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Active surveillance of fish and shrimp diseases was carried out in three districts of Tamil Nadu viz. Nagapattinam (South), Pudukkottai and Thanjavur for four years from 2013-2017. Passive surveillance was undertaken in five districts viz. Thoothukudi, Ramnad, Kanniyakumari, Tirunelveli and Madurai. The 560 shrimp samples collected were analysed for viral (WSSV, IHHNV, MBV, BP, TSV, YHV and IMNV), bacterial and parasitic pathogens. Sixty eight (68) WSSV (12.14%), 18 IHHNV positive samples (3.2%) were identified. Of the 346 samples analysed for EHP, 130 (37.57%) were positive. Targeted surveillance for AHPND in five districts including Thoothukudi and Ramnad since 2013 mainly by PCR (bacterial isolates tested=1121) and histopathology established freedom from AHPND. Infected fish samples were selectively screened for viral pathogens (SGIV, EHNV, IHNV, VHSV, SCVC, VNN, KHV, CyHV-2 and CEV) by PCR and cell culture isolation. Carp edema virus (CEV) was detected from koi undergoing mortality and the amplicon (547bp) sequence was submitted to Genbank. *Photobacterium damselae* subsp. *damselae* was isolated from infected cobia samples from cage culture. The bacteria was found to be pathogenic to seabass juveniles in experimental infection. *Aeromonas hydrophila* was isolated from *Pungasius* undergoing mortality in cages. From infected tilapia (GIFT), *Lactococcus lactis, Citrobacter freundii and Enterobacter* sp. were isolated. *Argulus, Lernaea*, gill fluke and *Trichodina* were found in various freshwater fishes. Eleven awareness programmes were conducted in 6 districts. A total of 574 farmers and department officials participated and benefited by these programmes. Nine pamphlets on shrimp diseases, BMPs in aquaculture and role of national surveillance programme in aquatic animal health management were distributed to the farmers.

ISAAHE-006

Surveillance of Pathogenic Organisms in Fisheries Products and Fish and Shellfish Diseases in Selected Districts of Kerala

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Increased intensification and expansion of aquaculture production has resulted in the emergence of new and transboundary diseases. This stimulated the role of disease surveillance programmes in the aquaculture sector. For the active surveillance programme, 1334 (finfish) and 344 (shellfish) samples were collected from selected 50 farms representing five districts of Kerala (Palakkad, Thrissur, Ernakulam, Alappuzha and Kottayam). 47 imported frozen finfish and 79 shellfish products were also screened for the presence of viral pathogens. Finfish were tested for KHV, SVCV; shellfish were tested for WSSV, IHHNV, YHV, TSV, AHPND, MBV, HPV and EHP. In this screening, all the finfish samples including products were tested negative for KHV and SVCV. WSSV, HPV, IHHNV, MBV was detected in 23.8%, 3%, 3%. 1.5% of the shrimp farms visited. YHV and TSV were not detected. Shellfish products were positive for WSSV (n=7), YHV (n=3) and MBV (n=1). During the entire period, 19 disease outbreaks were investigated in both shellfish and finfish aquaculture farms. Major cause for outbreaks were poor water quality, low dissolved oxygen level, high level of unionized ammonia (>0.5mg/L) and diseases due to infectious bacteria including *Aeromonas* sp. and *Edwardsiella tarda.* Pathogenic potential of these infectious bacteria were determined by infectivity studies. In case of shrimp, outbreaks were due to WSSV infection and poor water quality. Three training programs for officials from Kerala State Fisheries Department; six awareness programs for farmers and panchayat aquaculture coordinates were organized. Brochures on aquatic animals diseases, field guides and health cards were published and distributed.

ISAAHE-007

Surveillance of Freshwater Fish and Shellfish Diseases in West Bengal and Assam

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Survey and fish samples were collected from 40 selected fish and shrimp farms of West Bengal and 35 selected fish farms of Assam under active fish disease surveillance program. All the collected fish samples were screened for KHV, and SVCV using the specific primers and other bacterial pathogens using the 16S rRNA gene sequence analysis during the reporting period. The various bacterial pathogens viz. *Plesiomonas shigelloides* (KX986915), *Citrobacter freundii* (KX990295), (KP872908), KU613071, *Edwardsiella tarda* (KX998202), *Acinetobacter baumannii* (KT156752), *Acinetobacter lwoffii* (KT156753) and fungal pathogen viz. *Cladosporium tenuissimum* (KX999700) were isolated and characterized based on biochemical and molecular techniques from the diseased fish samples collected from West Bengal and Assam. The challenge studies were conducted for all those isolated pathogenic bacteria to confirm the Koch's postulate. Different parasitic diseases such as *Argulus, Myxobolus, Isoparorchis hypselobergi, Myxobolus, Diplostomum* spp., *Dactylogyrus and Lernaea* were found in the fish farms in West Bengal and Assam during the active surveillance. The shrimp samples (*L. vannamei*) were screened for EHP incidence in West Bengal and was found to be the most prevalent problem in shrimp farms. However, the *P. monodon* and *L. vannamei* were screened for WSSV and found positive in East Midnapur and North 24 Paraganas districts of West Bengal. ICAR-CIFRI, Barrackpore and CIFRI Regional Center, Guwahati organized 32 training-cum-awareness programs for fish farmers and state fisheries department officers in collaboration with state fisheries departments of West Bengal and Assam.

ISAAHE-008

Surveillance of Freshwater and Marine Fish and Shellfish Diseases in Andaman and Nicobar Islands

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Being one of the remotest sub-centres under NSPAAD, surveillance of freshwater and marine finfish and shellfish diseases is being conducted, covering all three districts of Andaman and Nicobar Islands (ANI). Baseline data has been collected from 161 freshwater fish farms and 23 fish landing centres. Two parasitic outbreaks of *Argulus sp.* and *Myxobolus sp.* were reported from freshwater fish farms and occurrence of branchial parasitic cymothoids namely, *Ryukyua circularis* from marine finfish, *Amblygaster sirm* and *Norileca indica* from carangid fish, *Selar crumenophthalmus* and marine super giant isopod, *Bathynomus lowryi* were reported for the first time from ANI. A total of 647 freshwater fish and prawn samples, 353 shrimp samples, 291 grouper samples and 20 goldfish samples were collected, of which all the samples gave negative result for the tested pathogens except IHHNV and WSSV. Shrimp samples (*Penaeus monodon*) collected from Lohabarrack, Betapur and Campbell Bay landing centres gave positive result for IHHNV. Likewise, shrimp samples *(P. monodon)* collected from Lohabarrack, Junglighat and Campbell Bay landing centres gave positive result for WSSV. To create awareness among the stakeholders, 11 awareness programmes, 3 training programmes and a sensitization workshop on NSPAAD were conducted at various places of ANI, in which a total of 402 participants including 349 farmers and 53 Fisheries Department Officers or Extension Officers were benefitted. Publications including technical bulletin (1), training manual (1), folders (3), pamphlet (1), proceeding (1) and Fish Health Card (1) were published in both English and Hindi version for the benefit of stakeholders.

ISAAHE-009

National Aquatic Animal Disease Database: A Dynamic Web Application

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Monitoring and surveillance of aquatic animal diseases is necessary for the prevention and control of aquatic animal disease on one hand and complying with the international disease reporting on the other hand for trade and commerce. Within the scope of the national project on aquatic animal disease surveillance, a disease database for documenting the aquatic animal diseases is developed.The present paper will give an overall different functionalities on architecture of the database for data capturing, analysis and reporting. The database is an online version with four different types of users: administrator, data entry operator, verifier and validator. Each users will accesses different modules depending upon hierarchy.User credentials with separate login ID and Password to each collaborating centers located at different states of the country were provided. This application is state of the art interactive software, supports data entry, data edit or management, verification, validation and reporting. Master tables for states, Districts, blocks and villages were created in addition to the master tables created on species, list of pathogens and exposures for Biological and outbreak samples. Query have been created to generate customized reports from the database. The important technologies used in the development of this applications are Dreamweaver, phpMyAdmin,Filezilla, PHP, MySQL, HTML, JavaScript, CSS, JQuery, Ajax, JSON, Bootstrap, Photoshop.

ISAAHE-010

Vannamei Diseases and Disease Surveillance in the State of Andhra Pradesh, India

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Andhra Pradesh is the "Aqua Hub" of India. Shrimp Diseases remain a concern principally because of the economic losses. The data collected during surveillance programme from five different districts of Andhra Pradesh viz., Srikakulam, Vizianagaram, Visakhapatnam, East Godavari and West Godavari reveal the preponderance of WSSV and emerging diseases like, EHP, White Gut and White Faeces are in the increasing trend. Under active surveillance, a total number of 455 samples is screened for different viruses viz., WSSV, IHHNV and microsporidian like EHP. Of these, WSSV (26.37%) is the major disease causing pathogen followed by EHP (13.84%) and IHHNV (2.63%). Apart from these pathogens, *Vibrio* (22.19%) is also causing damage to the shrimp farmers. White gut and white faecal matter (7.5%) are also posing considerable problems. Sample collection is in progress for emerging diseases like IMNV. WSSV disease outbreaks are more in East Godavari District (31.8%) followed by West Godavari District (18.2%), whereas EHP prevalence in East Godavari District is 14.1% and in West Godavari District it is 10.5%. In collaboration with Reliance Foundation Information Services, voice messages on Good Management Practices, Disease management are delivered to nearly 32,000 aqua farmers. A toll free Number 1800-425-1188 was arranged for all aquafarmers for timely technical guidance and extension services.

ISAAHE-011

Surveillance of Diseases in Fresh and Brackishwater Aquacultured Species in West Bengal

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Diseases of varied aetiology were, however a serious constraint to the success of many of the freshwater and brackishwater culture systems in West Bengal. Several bacterial, viral, parasitic fungal diseases, environmental and nutritional disorders have been documented. Prevention of these diseasesis accomplished through good water quality management, nutrition and sanitation. Without this foundation it is impossible to prevent outbreaks of opportunistic diseases.Successful fish health management begins with prevention of diseases rather than treatment. So surveillance is required for monitoring of the behaviour, activities or other changing information of fish/shrimp for the purpose of influencing, managing, directing, or protecting them. A fish/shrimp disease surveillance study has been initiated to develop a database on incidence of diseases and monitoring the emergent diseases of National concern. Accordingly, surveillance of 15 different cultured food fishes and 3 cultured shrimp/prawn species (economically important) and 10 different freshwater ornamental fish species have been made throughout Bengal from 2013 resulting in the detection of infectious and non-infectious diseases till date. Among the bacterial diseases Aeromoniasis, Vibriosis, and Edwardsiellosis were more prevalent. Among viral diseases, WSSV were of great importance in shrimp. The parasitic diseases of concern were Trichodiniasis, Myxoboliasis, Argulosis, Thelohanellosis, Dactylogyrosis and Ichthyophthiriasis which found both in IMC, catfishes and also in ornamental fishes. The different types of nutritional abnormalities observed in finfishes were lordosis, scoliosis, kyphosis and fish opercula deformity syndrome (FODS). These observations were recorded and put in soft form for development of disease database.

ISAAHE-012

Surveillance of Shrimp Diseases in Coastal Karnataka

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Since shrimp culture is rapidly expanding in Karnataka, it has become necessary to understand the pathogen associated during culture operation for the successful production. So we investigated the presence of pathogens in cultured shrimp in three coastal districts of Karnataka under the project "National Surveillance Programme for Aquatic Animal Diseases (NSPAAD)" since May 2013. Baseline data of 158 shrimp farms have been collected and samples were subjected to polymerase chain reaction based detection of DNA viruses such as white spot syndrome virus, hepatopancreatic parvovirus, monodon baculovirus, infectious hypodermal and hematopoietic necrosis virus and RNA viruses like taura syndrome virus, infectious myonecrosis virus, yellow head virus, microsporidian parasite like *Enterocytozoon hepatopenaei* and acute hepatopancreatic necrosis disease -causing *Vibrio parahaemolyticus* strain. Out of 209 shrimp sample pools analyzed since 2014, 49.2%, 3.34% and 0.9% sample pools were positive for WSSV, IHHNV and HPV, respectively. Among positive WSSV samples, 70% were positive by nested PCR; 3(5%) samples positive for EHP. Rest of the pathogens found to be absent in shrimps. To disseminate the findings of our study and best management practices (BMP) in farms, awareness programmes for shrimp farmers and state fisheries department officials were held. Our study result shows that WSSV is highly prevalent in aquaculture environment and shrimps are highly susceptible to WSSV. The present study strongly emphasizes the shrimp farmers to follow the best management practices (BMP) including strict biosecurity measures to get rid of pathogens in farms for the sustainable shrimp aquaculture in Karnataka.

ISAAHE-013

Surveillance of Freshwater Fish and Shellfish Diseases in Karnataka

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Surveillance of freshwater fish and shellfish diseases in Karnataka under National Surveillance Programme for Aquatic Animal Diseases was started in Karnatakawith a one day training programme on disease surveillance in aquaculture for the State Fisheries Department Officials. The main objectives of this project is to carry out baseline survey and active surveillance for Koi Herpes Virus and Spring Viraemia of Carp in six districts of Karnataka i.e. Mandya, Mysore, Shimoga, Raichur, Koppala and Bellary and also to report any outbreak of diseases in the freshwater ecosystem of Karnataka. The baseline data of 252 farms have been collected so far, while active surveillance has been carried out in 57, 61 and 81 farms during 2014-15, 2015-16 and 2016-17 respectively. On screening all the samples by PCR, it was found to be negative for KHV and SVCV. The disease case investigation was carried out in the district of Hassan, Dharwad and Shimoga. Considering the need for human resource development, a training programme and two awareness programmes were conducted for the Officers of Fisheries State Department and fish farmers. In addition to this pamphlet in Kannada on KHV, SVCV, EUS, disease surveillance, disease diagnosis and prevention were published to reach out to a larger mass of fish farmers.

ISAAHE-014

Status of National Surveillance Programme for Aquatic Animal Diseases in the State of Tripura

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Tripura is contributed significantly in both aquaculture production and consumption. National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) was launched in India with certain objectives and under this a subproject no. 22 entitled "Surveillance of Freshwater Fish and Shellfish Diseases in Tripura" was implemented during 2013. Under the programme both active and passive surveillance were being practiced and till date seven types of pathogens like *Argulus* sp., *Dactylogyrus* sp., *Myxobolus* sp., *Diplostomum* sp., infection with *Aeromonas hydophila*, *Aphanomyces invadans* and *Saprolegnia parasitica* were reported from the state. However, the types of pathogens being reported every year under the active surveillance were in decreasing order. During 2014-15 it was 7 and in 2016-17 it reduced to 4. Whereas under passive surveillance pathogens reported have increased from 3 to 6 numbers. Outbreak cases were decreased drastically under the active surveillance however, increased under passive surveillance. Collection of baseline data of farm was in increasing order since the inception of the project. During 2015-16, the baseline data collected was double the amount during 2014-15. Similarly, sample collection was almost three times higher in 2016-17 than the samples collected in previous years. On an average 5 awareness programmes were conducted every year and maximum during 2016-17. Physicochemical parameters of soil and water were also analysed and accordingly prescribed suitable management strategies. Thus, it can be concluded that surveillance programme is helping in reducing the outbreaks and on the other hand effective awareness programmes is strengthening the disease reporting system of Tripura.

ISAAHE-015

Surveillance of Fish and Shellfish Diseases in Maharashtra

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The active surveillance of fish and shellfish diseases in select districts of Maharashtra was undertaken from October, 2013 to March, 2017. The work was carried out under the aegis of National Surveillance Programme for Aquatic Animal Diseases implemented in the country by ICAR-National Bureau of Fish Genetic Resources, Lucknow. Altogether 39 fish/ shrimp farms of selected six districts in Maharashtra were targeted for farming and health management practices. Shrimp samples from selected farms were screened for WSSV, AHPND, EHP, IHNNV, MBV, IMNV using PCR assays as per OIE protocols. Awareness programmes were organised for shrimp farmers in Ratnagiri and for fish farmers in Kolhapur district. Among the OIE listed diseases of aquatic animals, WSSV and EHP were observed in *L. vannamei.* WSSV was predominant in all stages of shrimp. Out of 22 brackishwater farms screened for WSSV, shrimps in three farms (13.5%) were infected with WSSV in the year 2016-17. The figure showed an increased infection rate as compared to the previous years' data (8.33% in 2015-16 and 6.25% in 2014-15). EHP was noticeable during April-May period causing reduced growth in *L. vannamei* but mortalities were seldom observed. EUS was observed in *Puntius* spp. Argulus infestation was observed in Indian major carps in Dhom fish seed farms, Satara District. The cage cultured *Pangasius* spp. were prone to *Aeromonas hydrophila* infections. Fungal infestations in *Pangasius* spp. from *Saprolegnia* spp. in early fingerlings stages of growth was recorded in winter months.

ISAAHE-016

Surveillance of Freshwater Fish and Shellfish Diseases in Assam

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A total of five (5) nos. of districts viz. Nagaon, Morigaon, Sonitpur, Golaghat and Lakhimpur were to carry out the active surveillance programme. The baseline data from 63 numbers of farms were collected till March 2017. Under the active and passive surveillance programme 1505 nos. of fish samples were collected and made into 271 pools which were screeneed for various pathogens listed under the objective of the project. Under the passive surveillance, 61 nos. of different disease outbreak cases were recorded and have been screened for incidence of several fungal, bacterial and parasitic pathogen enlisted under the project as well as other etiological agents. Among all the pathogens *Aeromonas hydrophila* infection was recorded highest with a prevalence of 34.42 %, followed by the parasitic infestation of *Argulus* spp. with 26.22 %. The lowest incidence was recorded for leech infestation with 1.63%. During the implementation period of the project a total of 469 nos. of fisheries officers, farmers and entrepreneurs were targeted to bring awareness through awareness programme and training in fish disease diagnostics and management and its importance in fish farming system. An edited book for fisheries officials along with a farmers friendly booklet and three nos. of leaflets and a farmers visiting card were published under the project during the period.

ISAAHE-017

Surveillance of Fish Diseases in Coldwater Farming's of Jammu & Kashmir, Himachal Pradesh & Uttarakhand, India

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Database on fish disease surveillance in coldwater fish farms of Jammu & Kashmir, Himachal Pradesh & Uttarakhand was generated for a period April 2013-March 2017. Total 133 trout and carp farms/hatcheries from 1 district of Jammu & Kashmir, 4 districts of Uttarakhand & 2 districts of Himachal Pradesh were visited for active & passive surveillances. Cumulatively 1888 tissue samples were collected from 133 farms over the period. Tissue samples (426) from all the farms were pooled for the screening of viruses, bacteria, fungus and parasites. Tracking of VHSV, IPNV & IHNV confirmed negative by RT-PCR for the samples collected from trout farms of all three states. The general profiling of bacterial flora characterized in all the three states was *Aeromonas hydrophila, Aeromonas veronii* (*Ichthiosmia), Aeromonas allosachharophila, Pseudomonas fluorescence, Citrobacter freundii, Escherichia coli, Micrococcus*, *Acidovorax facilis, Enterobacter*, *Morganela, Hafnia alvei*, *Enterococcus gallinarum & Caronobacterium*. An outbreak of *Ichthyopthirius multifiliis* was recorded at early fall of 2016 & controlled successfully with salt application in Champawat, Uttarakhand. Acute outbreak of *Saprolegnia australis* on fertilized eggs of golden mahseer was recorded during summer 2016 at Bhimtal, Uttarakhand. Mass awareness camp (3nos), training programme (1no), demonstration cum interaction meet (1no) were organised involving all total 195 farmers in the state Himachal Pradesh & Uttarakhand.

ISAAHE-018

Surveillance of Shrimp Diseases in Gujarat

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Gujarat is having second largest coastal brackish water area in country. Shrimp farming is important commercial activity in Gujarat. Under National Surveillance Programme for Aquatic Animal Diseases, four districts namely Navsari, Suarat, Gir Somnath and Ahmedabad along with Diu (Union Territory) were selected. Total 1204 shrimp samples were collected from five districts of Gujarat from which 279 pooled samples were screened for important shrimp diseases. White spot syndrome virus positive shrimps had been observed from the shrimp farms of Navsari. *Enterocytozoon hepatopenaei*, a new parasitic infection was identified from the farms of Surat and Diu area. Vibriosis was found positive in some farms of Ahmedabad district. Apart from important diseases, problems such as white feces and black gill were also observed.

ISAAHE-019

First Report on the Occurrence of Infectious Myonecrosis Virus (IMNV) in Pond Reared *Litopenaeus vannamei* in India

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Whiteleg shrimp, *Litopenaeus vannamei* with clinical signs of white patch in abdominal muscle and muscle opaqueness with reddish colouration at the distal abdominal segments was observed in farms located in Purba Medinipur district of West Bengal state, India. The mortality of shrimp in all disease outbreak ponds ranged from 20 to 50% and increased gradually. The RT-PCR assay of these samples using different primer sets specific to infectious myonecrosis virus (IMNV) revealed the presence of IMNV in disease outbreak ponds. The IMNV infection was reproduced in healthy shrimp by intramuscular injection to satisfy River's postulates. The virus caused 73.33 to 100% mortality in intramuscularly challenged shrimp at 15 days post infection (d p.i.), but failed to cause mortality in orally challenged shrimp. Tissue distribution of IMNV in naturally and experimentally infected shrimp was studied by RT-PCR assay which revealed the presence of this virus in haemolymph, gill, hepatopancreas and muscle. The RT-PCR product amplified was sequenced and the results revealed 98% similarity with the sequence of IMNV. Histological examination showed coagulative muscle necrosis with haemocytic infiltration in the skeletal muscle of IMNV-infected shrimp. The present study confirms that the disease outbreak which occurred in the shrimp farms located at Purba Medinipur district, West Bengal, India was due to IMNV. This study forms the first report on the occurrence of IMNV in Indian shrimp culture system.

ISAAHE-020

An Outbreak of Mortality Associated with Betanodavirus in Asian Seabass (*Lates calcarifer*, Bloch) Cultured in Indoor Tanks and Open Sea Cages

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Viral nervous necrosis (VNN), also known as or viral encephalopathy and retinopathy, caused by Betanoda virus, is regarded as one of the most serious diseases of many farmed marine fishes in the world. The virus gains distinction due to the fact that it causes severe mortality in fries and juveniles and it can be transmitted vertically from brood stocks to offspring. We report an outbreak of mortality associated with Betanodavirus in Asian seabass fries reared in indoor cement tanks and juveniles reared in marine cages. In both the cases, the disease was characterized by clinical symptoms like abnormal swimming behavior which included circling, vertical swimming, etc, anorexia, white droppings, black pigmentation of body and mortality. Betanodavirus was confirmed by nested RT-PCR. While fish reared in indoor tanks showed 100% mortality, the mortality in cage reared fish was 30%. The survivors were positive to Betanodavirus till harvest. Grossly, the brain of ailing fish showed acute congestion while histologically moderate vacuolation of the grey matter was conspicuous.

ISAAHE-021

Marine Ranavirus (Similar Damsel Fish Virus, SRDV) Infection induces Immune Gene Expression in Freshwater Koi

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Vaccination is one of the best preventive measures against viral diseases in aquaculture. Adjuvants are essentially used for increasing the efficacy of vaccine formulations. Innnate defence mechanisms involving stimulation of pattern recognition receptors coupled with adaptive immune responses play an important role in the protection of the vaccinated animals against natural infections. In this study, inactivated cell culture grown similar damselfish virus (SRDV) vaccine was prepared and immunogenicity of the vaccine against virus infection was investigated in koi carp (*Cyprinus carpio*). The virus was formalin-inactivated and administered intraperitoneally along with Quil-A® adjuvant. The fish exhibited no clinical signs or lesions during the 28-day post-vaccination observation period. The expression profiles of the immune genes IRF-7 and IL-10 in spleen and kidney of the immunized koi supplemented with and without Quil-A® adjuvant were recorded post virus challenge. The IRF-7 expression level in the spleen of koi immunized with vaccine added Quil-A® adjuvant was highly up-regulated (52.2 fold) at 24 h post infection of the virus. In the kidney, it was showed down-regulation at 24 h post infection but the highest level of up-regulation (16 fold) was recorded at 96 h post infection of SRDV. Like IRF-7, highest up-regulation (331 fold) of the IL-10 was observed in the spleen of koi immunized with vaccine added Quil-A® adjuvant at 24 post infection while kidney was showed down-regulation. The study provides strong evidence for the existence of expression profiles of immune-related genes IRF-7 and IL-10 during the ranavirus infection in koi. It also suggests that Quil-A® adjuvant enhances the immune response of the vaccine candidates.

ISAAHE-022

Novel Chlamydiales Associated with Epitheliocystis in Farmed *Pangasianodon hypophthalmus* from Uttar Pradesh, India

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Pangasianodon hypophthalmus is a freshwater fish species belonging to the family Pangasidae. In India, the fish was introduced in 1995 and currently, it is cultured in monoculture as well as polyculture along with Indian major carps and pacu, *Piaractus brachypomus*. In the present study, diseased *P. hypophthalmus* (14-15 cm, 60-100g) with a history of continuous mortality were brought to Fish Health Laboratory by the farmer during July 2015. The wet mounts of gills revealed heavy infestation with trichodinids and monogeneans. The histological examination of gills from affected fish revealed the presence of granular basophilic cysts at the bases between the secondary lamellae which were typical of epitheliocystis. The presence of chlamydial DNA in affected gills was confirmed by amplification and sequencing of 16S rRNA gene. BLAST-n analysis of chlamydial signature sequence and near full length 16S rRNA gene from epitheliocystis agents revealed 96% similarity with *Candidatus* Actinochlamydia clariae. The phylogenetic analysis of the sequences revealed that epitheliocystis agents from *P. hypophthalmus* are novel and belong to taxa *Candidatus* Actinochlamydia. The present case is the first report of epitheliocystis from India, in a new fish host *P. hypophthalmus* and adds to the ever expanding range of hosts for these organisms.

ISAAHE-023

Shewanella putrefaciens Associated with Disease in Freshwater Fish *Oreochromis niloticus* in Hisar, India

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Shewanella putrefaciens, a gram-negative, motile, H_2S producing rod shaped bacterium belonging to the family Alteromondaceae, is the most important spoilage bacterium of iced fish and meat products. It is an opportunistic human pathogen and has been reported to cause bacteremia, skin and soft tissue infections. *S. putrefaciens* has also been reported to cause disease in freshwater fish under stressful conditions. In the present report, disease outbreak was observed in a fish farm culturing Indian major carps along with tilapia, *Oreochromis niloticus* in Hisar, Haryana during January 2015. Heavy mortalities were observed only in *O. niloticus* (Length 20-21cm, Weight 90-100g), which exhibited exophthalmia, erosions along with haemorrhages on the body surface and fin rot. On post-mortem examination, ascites and kidney liquefaction were observed. Histopathological examination revealed necrosis of renal tubules and haemorrhages in kidney, depletion of white and red pulp along with increase in melanomacrophage centres in spleen, haemorrhages in intestine and multifocal necrosis in hepatic parenchyma. Bacteria were isolated from blood and kidney of the affected tilapia and identified as *S. putrefaciens* on basis of biochemical tests and 16S rRNA gene sequencing. In addition, antibiotic sensitivity test revealed that isolated bacteria were sensitive to most of the antibiotics (n=21) but resistant to Penicillin, Teicoplanin and Bacitracin. The disease could be successfully reproduced in tilapia following intraperitoneal injection and bacteria could be re-isolated from experimentally infected fish, suggesting that *S. putrefaciens* was causative agent of disease outbreak in tilapia.

ISAAHE-024

Molecular and Biochemical Characterization of *Serratia marcescens* Isolated from Diseased Ornamental Fish, *Poecilia reticulata* in India

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During routine monitoring for diseases in ornamental fishes, a farmer reported heavy mortality (> 50%) in guppy, *Poecilia reticulata* with fin rot in June 2015. In bacteriological examination, two types of colonies were isolated on nutrient agar; red pigmented colonies which were predominant along with non-pigmented colonies. Red pigmented colonies were characterized and identified as *Serratia marcescens* (NPSM-1) on the basis of biochemical tests and partial sequencing of the 16S rDNA, and non-pigmented colonies were identified as *Bacillus* sp. Sequence analysis of 16S rRNA gene, metalloprotease gene and quorum sensing gene of *S. marcescens* showed that bacterial isolate, NPSM-1 had high sequence homology, ≥ 99% similarity with sequences of *S. marcescens* strains available in NCBI. A zone of lysis of sheep erythrocytes appeared around the *S. marcescens* colony on the blood agar after incubation for 12h. The bacterium was found to be susceptible to cefixime, chloramphenicol, ciprofloxacin, erythromycin and gentamycin out of 16 antibiotics. The ECPs from the NPSM-1 displayed cytotoxicity on Koi carp fin cell line. In experimental challenge, fish infected with NPSM-1showed clinical signs viz., darkening of skin, necrotic skin lesions and fin rot with 55% cumulative mortality and *S. marcescens* was re-isolated in pure cultures. The isolation of highly virulent and multi-drug resistant *S. marcescens*, a zoonotically important bacterium from freshwater ornamental fish, poses a threat to the farm personnel. Further epidemiological investigations together with studies on *S. marcescens* pathogenicity are necessary to elucidate the public health significance of *S. marcescens*.

ISAAHE-025

Histopathological Investigation of Cultured Shrimp Diseases in Coastal Regions of Odisha in India

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A shrimp disease survey was conducted in selected coastal regions of Odisha in India during the period of one year from May 2014 to April 2015. The objective of this study was to acquireinformation regarding the diseases prevalent in shrimp culture farms. Shrimp samples were collected from ponds exhibited disease symptoms and also from ponds reported to be in good health. Both *Penaeus vannamei* (n=61) and *P. monodon* (n=41) samples were subjected to the study by histopathological examination. Hepatopancreatic Microsporidiosis (HPM), White spot disease (WSD), Septic Hepatopancreatic Necrosis (SHPN), lymphoid organ spheroids (LOS) and external fouling organisms (EFO) were found to be common in both shrimp species, whereas, Monodon baculovirus (MBV) and Hepatopancreatic parvo-like virus (HPV) were detected only in *P.monodon*. In this study, a high prevalence of HPM was detected in 23% of the farms followed by LOS (20%), SHPN (18%), MBV(18%) and EFO (17%). However, comparatively less prevalence was observed for WSD (4%) and HPV (1%). The significance and implications of these findings for the shrimp aquaculture industry in India are discussed.

ISAAHE-026

A Pilot Surveillance Status of Infectious Myonecrosis Virus in Indian Shrimp Aquaculture

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Disease surveillance programmes provide information on diseases that miligate against aquaculture production. Infectious myonecrosis virus (IMNV) has emerged recently as one of World Animal Health Organization (OIE) listed viral diseases in penaeid shrimp, caused by dsRNA virus. The present study was conducted to investigate the prevalence of IMNV along the Indian coastal states. Samples of Pacific whiteleg shrimp, *Litopenaeus vannamei* and Green tiger shrimp, *Penaeus monodon* were collected from 21 randomly selected active shrimp farms located along the coast by using a risk-based two-stage random sampling survey. Screening of the sampled shrimps for the presence of IMNV based on nested RT-PCR diagnostic tool, revealed that IMNV was not detected in all the samples examined. The present investigation can shows that IMN or IMNV was free from shrimp aquaculture along Indian coastal states. Further studies can be conducted on the disease modeling and active target of this virus on national wide surveys.

ISAAHE-027

Association of *Aeromonas veronii* biovars in the Mortality of Ornamental Goldfish

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Motile aeromonads are the most significant bacterial pathogens which play a major role in causing diseases in freshwater ornamental fishes. The study was conducted to evaluate the pathogenicity of *Aeromonas veronii* biovars isolated from infected ornamental freshwater fish. A total of 68 bacterial isolates were identified as genus *Aeromonas* from 123 investigated samples and were further identified up to complex level based on four biochemical tests and upt o species level using Aerokey II and Moeller decarboxylase and dehydrolase reaction. These tests helped us in distinguishing *A. veronii* biovar veronii and *A. veronii* biovar sobria. The molecular identification using 16S rRNA sequencing validated the results of biochemical identification. Goldfish, the experimental animal was challenged with the isolated bacteria to explore the virulence pattern. In the study *A.veronii* biovar sobria was found to be more virulent compared to A.veronii biovar veronii with LD_{50} value of 1.74×10^5 and 2.6×10^6 cfu/ml respectively. Most of the isolates exhibited marked haemolytic, proteolytic, caseinase, nuclease and crystal violet activity. They also exhibited pronounced congored uptake, slime production, biofilm formation, cell surface hydrophobicity, and suicidal activity. Polymerase Chain Reaction was carried out to investigate the virulence markers present in the isolates. A. veronii biovar veronii isolate along with the reference strain harboured all other genes except TapA. Ast gene was lacking in *A. veronii* biovar *sobria*. Antibiotic susceptibility test revealed the resistance of the isolates to penicillin-G, Ampicillin, Ticarcillin, Amoxyclav, Carbencillin, carbapenem, tetracycline, steroids, fluroquinolones, polypeptides, trimethioprim. The present study confirms thevirulence potential of both the isolates and *A. veronii* biovar. sobria was found to be more virulent than *A.veronii* biovar veronii in goldfish.

ISAAHE-028

Vibrio parahaemolyticus Infection in Diseased Black Tiger Shrimp *Peneaus monodon*

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Diseases caused by *Vibrio* spp. have direct effect on production byreduced feed intake and growth rate which may limit development of shrimp aquaculture industry. Among the *Vibrio* spp., *Vibrio parahaemolyticus* is an important cause of disease, mortality and economical loss in the shrimp culture development. In the present study, we have investigated a case of disease outbreak in black tiger shrimp juveniles *Peneaus monodon* from a commercial aquaculture farm in Kerala, India.The bacterial species responsible for the disease were identified as *V. parahaemolyticus* based on traditional biochemical methods and by PCR. All the isolates were negative for PCR specific for *V. parahaemolyticus* strains associated with acute hepatopancreatic necrosis disease (AHPND). Experimental infectivity studies using *P. monodon* post larvae by immersion challenge with eight selected strains of *V. parahaemolyticus* from the moribund and apparently healthy shrimps showed that the LD_{50} value of the isolates ranged from 5.06 X 10^4 to 3.9 X 10^5 CFU mL^{-1}. BOX PCR of the bacterial strains followed by dendrogram analysis by Gelcompare II software revealed that the strains isolated from moribund and apparently healthy shrimps formed two separate clusters indicating the origin of these strains are from separate clones. These findings will significantly increase our understanding of the disease caused by *V. parahaemolyticus* as it is necessary to develop new control strategies for Vibriosis in shrimp aquaculture.

ISAAHE-029

Morphological and Molecular Characterization of *Saprolegnia* sp. Infecting Different Life Stages of Rainbow Trout and Golden Mahseer from Himachal Pradesh and Uttarakhand (India)

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In the present study, Saprolegnia species, an economically important group of oomycetes were isolated from diseased rainbow trout farms of Himachal Pradesh and from hatcheries of golden mahseer. Characterization of fish pathogenic *Saprolegnia*is done for the furtherance of epidemiological studies of the source of infection, disease transmission and disease spreading. Ten rainbow trout farms of district Mandi of Himachal Pradesh and golden mahseer hatcheries of Bhimtal, Uttarakhand were surveyed for oomycetes infections. The cumulative mortalities in rainbow trout and golden mahseer was more than 10-15% in farms and hatcheries. The diseased samples showing sign of cotton wools and heterogeneous growth were taken for identification and further characterization. The morphological characterization of *Saprolegniasp* was based on predominance of diclinous antheridia branches and the DNA of the cultured oomycete was extracted for further investigation.Genotypic characterization was done using internal transcribed spacer (ITS) universal primer. ITS 1 and ITS 4 were sequenced. Further, sequence similarity was performed using NCBI BLAST online application. The oomycetes from eggs of golden mahseer, *Tor putitora* and adult rainbow trout, *Oncorhynchus mykiss* belonged to the genus *Saprolegnia* and were identified as *S. diclina, S. australis* and *S. parasitica.* The present scrutiny could supplement the information to the scientific community regarding the pathogenicity, prevalence and taxonomic ambiguity which can effectively be resolved using ITS molecular sequencing.

ISAAHE-030

Aeromoniasis and Argulosis in Freshwater Fish Farms of Southern Odisha

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The present work on prevalence of fish diseases in Southern Orissa revealed the occurrence of Aeromoniasis and Argulosis. For investigative purpose five different districts namely Ganjam, Gajapati, Kandhaman, Kalahandi and Rayagada were selected considering the potential of fisheries and aquaculture resources. More than 1500 fishes were sampled for analysis during the entire study period of 2014-2015, 2015-2016 and 2016-2017. The bacterial and parasitic pathogens were found to be the major agents of disease in Indian Major Carps and other cultured fish species. The isolated bacterial species were identified as *Aeromonas* spp., *Flavobacterium* sp., *Pseudomonas* sp. etc. Whereas, the isolated parasites were identified as *Argulus* sp., *Dactylogyrus* sp., *Thelohanellus* sp. etc. The bacterial species were found from the kidney, gill and skin samples, whereas, parasites were found from gill and skin samples of the affected fish. Externally, fishes exhibited loss of scales, haemorrhages, fin and tail rot, gill whitening etc. and internally, fishes showed discolouration of internal organs with fluid accumulation in the samples of diseased fish. Histopathological observations of gill, liver, spleen and kidney associated with bacterial infections revealed the disease development processes. During the study period, Aeromoniasis and Argulosis showed the highest severity index and prevalence in winter months than the summer. The fish farms of Kalahandi district were found to be more susceptible to Aeromoniasis and Argulosis followed by fish farms of Ganjam, Rayagada, Gajapati and Kandhamal districts. Monitoring of such disease scenario in freshwater fish farms of districts in Southern Odisha, will help the researchers, administrators and the fish farmers to meet the challenges arising due to disease outbreaks.

ISAAHE-031

Incidence of *Isoparorchis hypselobagri* – A Trematode Parasite in Fishes of Kalong River in Assam

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A study carried out in the Kolong river of Nagaon district, concluded the incidence of a *Isoparorchis hyselobagri*, a digenetic trematode parasite infecting bottom dwelling fish species. *Mastacembalus armatus, Notopterus noptopterus and Wallago attu.* The highest prevalence was found in the fish species *M. armatus* followed by *N. notopterus* and *W. attu*. The detailed pathology of this parasite in fish host was not well understood, but formation of cysts and black discoloration of the site of infection in host fish species infected by *I. hypselogabri* were observed to be very prominent. Immature forms of the parasite were found in different body parts of *M. armatus* amd *N. notopterus*, but no mature parasite were recovered from these two fish species. The mature forms of the parasite were only recovered from the swim bladder of silurid fish *Wallago attu.* Since both the parasites were confirmed to be the same using DNA assay, indicating that the immature forms of the parasites get maturity in the swim bladder of *wallago attu*.

ISAAHE-032

Occurrence of Multiple Infection Caused by White Spot Syndrome Virus (WSSV) and *Enterocytozoon Hepatopenaei* (EHP) in Pond Reared *Litopenaeus vannamei*, and Standardization of A Multiplex PCR Protocol for their Simultaneous Detection

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White spot syndrome virus (WSSV) is a devastating shrimp viral pathogen. Next to the WSSV, a new microsporidian parasite namely, *Enterocytozoon hepatopenaei* (EHP), responsible for stunted growth in *L. vannamei* has been reported in different parts of India. The NFDB funded surveillance programme carried out in the present study revealed the presence of multiple infection caused by WSSV and EHP in the samples collected in the month of November, 2016 from four farms located in Kanchipram district, Tamil Nadu State. The size of shrimp ranged from 11.8 to 14.2 g with age of 92 days of culture. The mortality due to multiple infection in all the ponds ranged from 39.7 to 46.4%. Due to heavy mortality, the culture was terminated in all the ponds. The clinical signs observed include stunted growth, lethargy, anorexia and minute spots at the carapace and first and second abdominal segments. The PCR analysis was carried out separately for the samples from these four ponds for WSSV, IHHNV, EHP and AP4 for EMS and the results revealed the presence of WSSV and EHP in all the four samples. A multiplex PCR protocol was standardized for simultaneous detection of WSSV and EHP. Various parameters such as concentration of DNA, annealing temperature, primer concentrations, number of cycles and annealing elongation time were optimized for simultaneous detection of WSSV and EHP. This multiplex PCR protocol helps to detect these pathogens simultaneously in one step in a single tube and would be cost effective and time saving.

ISAAHE-033

Isolation and Characterization of Bacteria Associated with EUS infected *Channa Striata (*Bleeker,1879)

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Diseases like epizootic ulcerative syndrome (EUS), hemorrhagic septicaemia, fin rot, tail rot, gill rot, dropsy etc. are caused in fish due to bacterial pathogens. The present investigation was carried out from October 2016 to March 2017 to isolate and characterise bacterial pathogens obtained from the naturally infected fish, *Channa striata* (a freshwater snakehead) collected from Deepor beel (a wetland) of Assam, India. Fish showed lethargic movement, severe infection in the head and tail region. Five different types of bacterial colonies were obtained when swab was collected aseptically from the infected region and was inoculated in nutrient agar medium. Colony morphology and a number of biochemical tests were carried out for identification of these bacterial strains, suggesting the microbial community were appeared to be dominated by *Bacillus* and *Aeromonas* sp. Both the *Bacillus* and *Aeromonas* isolates were further identified via use of PCR technique using universal 16SrDNA primer.

ISAAHE-034

Screening of Farmed Fish of Bihar for Koi Herpes Virus and Spring Viraemia of Carp Virus

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Surveillance programme was initiated under the project "National Surveillance Programme for Aquatic Animal Diseases" in five districts of Bihar namely Patna, Vaishali, Samastipur, Darbhanga and Madhubani. Biological samples like liver, kidney and gill tissues were collected from various fish ponds across different districts. Samples were brought to the laboratory for further analysis. For Koi Herpes Virus (KHV) detection, DNA was extracted from fish tissue using Phenol Chloroform Isoamyl alcohol method followed by its PCR amplification and gel electrophoresis. Similar procedure was followed for detection of Spring Viraemia of Carp Virus (SVCV) involving additional steps of RNA extraction, cDNA synthesis and lastly PCR amplification. The *in vitro* amplification for both the virus genome were done along with a positive and negative control to rule out false positive result. The present study indicates that, none of the sample is PCR positive for KHV and SVCV. This indicates that, fish stock of different districts under investigation are not infected with these two virus till date. However, many samples were infected with ectoparasites like *Argulus* sp., *Lernaeae* sp. And bacteria predominantly *Aeromonas* sp. The ectoparasites collected from fields were subjected for DNA barcoding using cytochrome oxidase subunit I (COI) universal primer for species level identification and characterization.

ISAAHE-035

Potential Whirling and White Spot Disease in Rainbow Trout Fry and Fingerlings: Their Impact & Management

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Present study described two important parasitic outbreaks from fish farmer's raceways and trout farms of Uttarakhand along with the impact on fish health & their control through suitable management measures. Initially, whirling movement was observed in 35- 40 days old trout fry with average size of 38.2±5.9mm and 46.6± 2.37 mg during indoor rearing. The clinical sign recorded for whirling movement were retarded growth, blackening of caudal area, curved tail, presence of a cyst like growth in the peduncle area and hemorrhagic gills. Mortality of fry was ranged from 5 to 25% over the 110-125 days of rearing period. With decline of water temperature, the severity of infection was reduced. In outdoor nursery raceways, the infection of *Ichthyophthirius multifiliis* was recorded in trout fingerlings with an average size and weight of 118.06 ± 9.67mm and 20.61± 4.86 g. Presence of small white nodules on body surface, raised fins, black coloration of body, excessive mucus secretion, with anorexic conditions were the external clinical signs exhibited by infected fingerlings. The degree of infections was positively correlated (r = 0.98) with increasing in water temperature (>19.5^{0}C). Mortality of fry was ranged from 3 to 7% over and observation period of 15-20 days. The maximum prevalence along with mean intensity of *I. multifiliis* infection was 36.66% & 3.44 respectively. A successive (7 days) dip treatment trial of 2% NaCl for 30 second, reduction in stocking density & regular cleaning of the raceways were found effective in controlling the parasitic infections.

ISAAHE-036

Evaluation of Stability of Reagents of Nano Gold Labelled Monoclonal Antibody for Flow-Through Assay (Immunogold Test)

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White spot virus (WSSV) is a stumbling block to shrimp culture world over. A farmer level monoclonal antibody (MAb) based flow through immunogold kit was developed in our laboratory for detection of WSSV. The kit is very simple to farmer level, requires only 2 min for completion and is 100 times sensitive than 1st step PCR. However, considering transport and storage conditions there was a need to improve the stability of colloidal gold conjugated MAb. In the present study, the shelf life of monoclonal antibody (C-05) based Immunogold kit at different storage buffers (10mM PBS ; 10mM PBS + 0.05% sodium azide ; 10mM PBS + 20% glycerol + 0.05% sodium azide) and temperatures (room temp., fridge (4-8^{0}C) and freezer (-20^{0}C)) was evaluated for 3 months for detection of WSSV. It was found that kit was stable upto 90 days at all the three different storage buffers and temperatures but intensity of the dots of kit stored at room temperature decreased form 15th day to 90th day. Among the three, kit prepared with gold labelled monoclonal antibody in storage buffer (10mM PBS and 0.05% sodium azide + 20% glycerol) at fridge temperature had showed better results and it is most suitable for the common farmer, also, it is cost effective.

ISAAHE-037

Development of Polyclonal Antibody Based Immunodot and Flow Through Immunoassay for Detection of *rPirA* Protein Causing Acute Hepatopancreatic Necrosis Disease (AHPND) in Shrimp

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Early mortality syndrome (EMS)/Acute Hepatopancreatic Necrosis Disease (AHPND) is currently the most significant disease in shrimp farms in Vietnam, Thailand, Malaysia, China and Mexico, and there is a great risk that it may spread to other shrimp farming countries. While there are assay of sophisticated detection tools for the detection of AHPND, a need for a sensitive, simple and rapid detection method still persists on these grounds antibody based assay are gaining much attention. In the present study, a simple, sensitive, rapid, farmer friendly flow through assay for the detection of AHPND pathogen has been developed. The rabbit antiserum produced against *rPirA* protein is strain specific causing AHPND pathogen. Rabbits were immunized (IM) with recombinant protein at 21 day intervals and antibody titre was determined by ELISA. The highest titer was observed at 3rd booster. The raised rabbit antiserum was characterised, no cross reactivity with same species of AHPND free *Vibrio parahaemolyticus, V. anguillarum* and other pathogens such as purified WSSV, *Aeromonas hydrophila* and *Aphanomyces invadans*. Using the same antiserum, Immunodot and flow through immunoassay (FTA) were developed. The developed tests, specifically showed reactivity with purified *rPirA* and not with other Pathogens. And, FTA could detect rPirA at 0.121 μg/ml compared to 0.485μg/ml by immunodot assay. FTA required only 10min for completion compared to 3 h by immunodot and both the assays were cost effective. Among, FTA is a better hope towards the real-time management decisions regarding emergency harvest would be possible for the prevention of AHPND.

ISAAHE-038

High Level Recombinant Betanodavirus RNA2 Coat Protein Expression in Prokaryotic System: For Production of Subunit Vaccine and Immunodiagnostics

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Viral Nervous Necrosis, a viral disease of fish caused by Betanodavirus, results in serious mortality and economic loss to the aquaculture industry. Presently, in the absence of any specific treatment, managemental measures like stocking of virus free seedlings and prophylactic vaccination are the only solutions to combat the infection. RNA2 coat protein of betanodavirus is an ideal candidate for vaccine development. RNA2 coat protein gene was isolated from VNN infected open sea cage reared Asian seabass (*Lates calcarifer*). The gene was used for producing recombinant RNA2 protein in a prokaryotic expression system. The expression conditions were optimised using an autoinduction system to obtain high level expression (51mg/l of culture) of rRNA2. The purified protein will be useful in developing Recombinant Vaccine against betanodavirus. The recombinant protein can also be used for the development of immune assays, including Lateral Flow assays for detection of betanodavirus.

ISAAHE-039

Development of Highly Specific and Sensitive Nested PCR Primers for Detection of Herpesviral Haematopoietic Necrosis Virus in Goldfish, *Carassius auratus* (Linnaeus, 1758)

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Cyprinid herpesvirus has made its way into scientific research due to its pathogenicity and the economic losses they cause to the aquaculture industry. The genus *Cyprinivirus* contains four species: cyprinid herpesvirus 1, cyprinid herpesvirus 2 (CyHV-2), cyprinid herpesvirus 3 and anguillid herpesvirus 1. CyHV-2, also known as Goldfish herpesvirus (GHV) and Herpesviral Haematopoietic Necrosis virus (HHNV), has been reported from many countries like Japan, China, USA, Australia, New Zealand, Taiwan, UK and France. Recently, mass mortality of goldfish due to CyHV-2 infection has been reported in India. The diagnosis of CyHV-2 infection is carried out using primers targeting the DNA polymerase and helicase gene fragments. During latent stage, the CyHV-2 detection appears to be difficult by PCR. In our study, we have designed nested primers targeting amplification of 932 bp and 331 bp fragment of the major capsid protein (MCP) gene of CyHV-2, using the sequences (Accession No. KM200722 and NC019495). The specificity of the new primer was confirmed with other viruses in the genus viz., Cyprinid Herpesvirus-3, Spring Viraemia of Carp Virus, Iridovirus and carp edema virus and they gave no amplification in PCR. The primers in present study were able to detect repeatedly 0.01 pg of CyHV-2 DNA , whereas, the DNA polymerase could detect up to 1 pg of DNA. These results suggest that the designed primers from MCP gene are highly specific as well as sensitive for detecting CyHV-2, and are ideal for detection of virus in latently affected fish.

ISAAHE-040

Development and Validation of Loop Mediated Isothermal Amplification for the Detection of *Enterocytozoon hepatopenaei*

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Enterocytozoon hepatopenaei (EHP), emerging microsporidian parasite, has been widely reported in several shrimp farming countries in Southeast Asia. Since 2015, the prevalence of EHP has been found to be significant (31% of 185 farms investigated), especially in the states of Tamil Nadu and Andhra Pradesh. EHP was associated with 14% of WSSV cases, 28% of size variation, 30% of white faeces syndrome and 11% of white muscle syndrome. EHP can be detected by PCR targeting 18 s small subunit (SSU) rRNA or spore wall protein genes (SWP), using extracted DNA from hepatopancreas tissue, faeces and whole post larvae. In this study a rapid sensitive and specific, loop mediated isothermal amplification (LAMP) protocol was developed for diagnosis of EHP. LAMP primers were designed based on the 18s (SSU) rRNA gene of EHP. The amplification was carried out at constant temperature of 65°C for 45 min and amplified LAMP products were visually detected in a closed tube system using SYBR green dye. The sensitivity of this LAMP protocol was found to be 10 copies. Field and clinical applicability of this assay was demonstrated with 152 field samples including 96 hepatopancreas samples, 56 shrimp faecal samples collected from shrimp farms, from which EHP could be detected in 55 samples. This LAMP assay is rapid, specific and sensitive than SSU-PCR and equivalent to nested spore wall protein (SWP) PCR. This tool could be used for studying the life cycle, transmission and involvement of intermediate hosts, besides having scope for on farm use.

ISAAHE-041

Monoclonal Antibody Based Diagnostics to Commonly Used Antibiotics in Aquaculture

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Owing to its extreme nutrition value and significant contribution to global food security, aquaculture is considered as one of the most important economically impactful food production sector in the world. Intensification in aquaculture leads extensive use of antimicrobials to combat with the diseases, caused huge economical loss to this industry. Extensive use of antibiotics without maintaining proper withdrawal period often leads antibiotic residues in fish tissues. Besides its adverse effect on the aquatic microflora and accelerating the process of development of antibiotic resistance, antibiotic residues harm human health by causing liver toxicity, gastrointestinal and photosensitive allergic reactions. Therefore detection of these drug residues are utmost important. Currently used analytical methods such as LCMS/MS, HPLC, ELISA are sophisticated laboratory lased laborious tools. In present work, we developed monoclonal antibody based immunoassays (membrane based) to detect two commonly used antibiotics i.e. oxytetracycline and sulphadimethoxine in culture fisheries. The assay could be accomplished within ten minutes. Reliability of developed technique was co-evaluated with HPLC and validated using field fish samples from different regions of southern India. The developed low cost antibody based immunoassay provides high sensitivity (far below their MRL level) with rapidity which could definitely meet the requirements of field level analysis.

ISAAHE-042

A Single Tube Reverse Transcription– Loop-Mediated Isothermal Amplification (Single Tube RT-Lamp) for the Detection of Betanodavirus Infection

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Viral Nervous Necrosis (VNN), also known as Viral Encephalopathy and Retinopathy (VER), is caused by betanodavirus in larvae and juveniles of marine fin fish, resulting in mass mortality. No effective treatments are available and fish surviving the infection remain carriers of the virus, facilitating vertical and horizontal transmission. Hence only preventive measures like vaccination, regular screening of broodstock, eggs, larvae and fingerlings besides effective disposal of positively diagnosed specimens are options available for the containment of disease associated with betanodavirus in hatcheries and aquaculture farms. A single tube Reverse Transcription – Loop – mediated Isothermal Amplification (single tube RT-LAMP) was developed for the detection of Betanodavirus infection. The developed method is highly specific, sensitive, rapid, and can detect a single copy of virus in less than one hour. Positive reaction is identified by a green fluorescence that can be visualized by naked eye under visible or UV light (using protective goggles) so that sophisticated equipments like a thermal cycler or trans-UV illuminator are not required. Mainly intended to screen marine brood stock fish to ensure certified specific pathogen free eggs and larvae in a sensitive and rapid way, the developed method also aids in timely diagnosis and prevention of spread of disease in marine fish culture system.

ISAAHE-043

Development of Monoclonal Antibody based Marker for Quantification of Macrophages in Blood and Lymphoid Organs of *Labeo rohita* (Hamilton)

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Monoclonal antibodies (MAbs) to cell surface molecules are important tools to identify and quantify different leukocytes. In the present study, A9 MAb was raised against macrophages using purified populations of rohu *Labeo rohita* peritoneal macrophages (LRPM) as the immunizing antigen. A9 MAb specifically detected a 62.2 kDa polypeptide of the LRPM membrane proteins and did not show cross reactivity with rohu serum proteins. This MAb was found to be suitable for the detection and quantification of macrophages in blood and lymphoid tissues of rohu. For this, mononuclear cells (MNCs) from blood, and lymphoid organs were stained by A9 MAb and analyzed by means of flow cytometry. Analysis of gated cells presumed to be macrophages revealed that the percentage of A9+ cells were 51.37% (blood), 96.55% (kidney), 3.06% (spleen) and 41.99% (thymus). Slight cross reactivity to kidney granulocytes was also seen as ~9% of gated granulocytes in kidney were also immune stained by A9 MAb. The anti-rohu macrophage MAb could detect macrophages in sections of kidney, thymus and spleen; and also in blood smears by the indirect immune peroxidase test. These results suggest that A9 MAb can be a suitable marker for detection and quantification of rohu macrophages.

ISAAHE-044

Studies on the Sensitivity of Immunodetection of WSSV Using Single or Panel of Monoclonal Antibodies

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With WSSV continuing to hail to be the notorious shrimp virus causing multimillion dollar loss to the shrimp industry, its early detection and eradication becomes pivotal. Although a multitude of sophisticated WSSV detection kits and tools have been designed, there is always a scope for developing a simple, effective and farmer friendly detection kits. Addressing this issue in the present study, a panel of 4 MAbs (C-05, C-14, C-38 and C-56) specific to VP28 developed were used to formulate MAb cocktail and used in the flow through assay-RapiDot in order to enhance the sensitivity of detection of WSSV. Recombinant VP28 was used to standardise this immunodetection and further analysed on semi purified WSSV.The detection limit of WSSV by MAb cocktail was found to increase by two fold relative to the single MAb currently being used in RapiDot. Out of the total 15 MAb combinations, the 2 MAb cocktails C-05+C-56 and C-14+C-56 were found to be the most suited for the assay. They exhibited highest sensitivity in the detection of WSSV and requires only 2 MAbs to formulate them. On comparing the immunodetection with PCR, the MAbs cocktail was 100 times more sensitive than 1-step PCR and nearly equivalent to 2-step PCR.

ISAAHE-045

Molecular Characterization and Pathogenicity of A Virulent *Plesiomonas shigelloides,* Associated with Mortality of Farmed Indian Major Carp *Labeo rohita* (Hamilton 1822)

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Indian Major Carp *Labeo rohita* (Ham.) is the most preferred cultivable fish species under freshwater aquaculture in India. The present study was conducted to characterize the bacteria at molecular level and to understand its pathogenicity associated with mortality of farmed rohu, *Labeo rohita*. Diseased fish samples were collected from North 24 parganas, West Bengal, India for the isolation of pathogenic bacteria. The 16S rRNA gene sequence of the bacteria revealed that the isolate was 100% identical with *Plesiomonas shigelloides.* (NCBI Accession number KX986915). Intraperitoneal injection with the isolate at the level of 1.5 X 10^6 CFU/ml in fish causes mortality. The challenged fish had loss of mucus and reddish lesion near the pectoral fin, however there was no sign in the gill. Histopathological studies revealed that damaged glomerulus in kidney and necrosis of hepatocytes in liver of diseased *L. rohita*. Understanding the pathology, pathogenesis and antibiotics resistant mechanisms of this emerging pathogen in cultured carps could help in management of the diseases.

ISAAHE-046

Purification, TEM and Cloning of Betanodavirus of Finfishes

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Betanodavirusis a potential pathogen of many marine and brackish water fish species. It has been reported from freshwater fishes also. Purified viral particles are useful for immunodiagnostics and for vaccine development. In this study, purification of betanodavirus was attempted through ultracentrifugation using CsCl density gradient. SSN-1 cells were infected with betanodavirus and after recording CPE cells were harvested. Virus infection was confirmed by PCR assay using reported primers and further sequencing of cloned cDNA. Transmission electron microscopy was carried out for confirmation of infection. Viral particles from cell culture supernatant were purified using $CsCl_2$ density gradient and ultracentrifugation. Purified virus was further tested for infectivity by incubating with SSN-1 cell line for its biological activity.

ISAAHE-047

Development of Farmer Level Rapid Immunoassay for Determination of Immunoglobulins Level in Farmed and Wild *Catla Catla* (Hamilton)

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A thorough understanding of fish immune system is a basic necessity in effective management of fish diseases in aquaculture.Studies on carp immune system are limited even though carps form the mainstay of Indian aquaculture. Determination of immune molecules such as fish serum immunoglobulin levels has been used as a general parameter to asses' immune competence in vaccination, disease management and also to evaluate possible effects of environmental conditions on the immunological status of a fish population.At present, simple and rapid assay to detect and determine the immune status of carps is not available. In the present study, for the first time, monoclonal antibody based farmer level rapid immunoassay was developed for determination of Ig level in farmed and wild *Catla catla*. Monoclonal antibodies (MAbs) were raised against the immunoglobulin (Ig) of Indian Major Carp (IMC), catla. Two clones C6E2 and C6F7 were selected for characterization and development of immunoassay. Both the MAbs reacted with the heavy chain (85 kDa) of the Ig in Western blot. MAb C6E2 and C6F7 could detect the purified Ig up to 16 μg ml^{-1} in the immunodot assay. The MAbs could detect the Ig in the unimmunized crude serum till 1000 dilutions of the serum. Further standardisation and development of flow through assay resulted in detection of the Ig up to 1:50 dilution of the serum by both the MAbs. The developed kit can be used in determining the level of total Ig and also for evaluating and developing effective vaccines for catla.

ISAAHE-048

Rapid Detection of White Spot Syndrome Virus (WSSV) of Shrimp by Field-Usable Lateral Flow Immunoassay

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White spot syndrome virus (WSSV) is one of the important shrimp viral pathogens. The detection of WSSV depends mainly on molecular diagnostic tools like PCR and real time PCR. These tools are neither field-usable nor rapid enough considering the speed at which the virus spreads. Therefore, development of a rapid, reliable and field-usable diagnostic method for the detection of WSSV is imperative to prevent huge economic losses in the culture system. Based on our previous study, a lateral flow immunoassay (LFIA) was developed to detect this killer virus which would be helpful to shrimp farmers and hatchery operators for screening seed and brooders to avoid the WSSV infection in the culture system. The development of LFIA strips comprised of three steps, viz. (a) production of polyclonal antibody against WSSV, (b) synthesis of colloidal gold nanoparticles-antibody conjugate and (c) assembly of LFIA strips in polypropylene cassettes. The sensitivity and specificity of LFIA were tested, and the sensitivity was compared with that of single step PCR and real time PCR. The LFIA was able to detect WSSV from hemolymph as well as gills of an infected animal at 6 hour post infection (h p.i.). The LFIA was used to detect the virus in the field samples collected during surveillance programme. The PCR detected WSSV in 45 samples and LFIA in 44 samples out of 75 farm samples. The LFIA has an excellent potential for use in the field and could prove to be a boon to the shrimp culture industry.

ISAAHE-049

Comparative Analysis of *Vibrio* Isolates in WSSV and EHP Infected and Uninfected Shrimps with Reference to Plasmids and Antibiotic Sensitivity

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Shrimp is one of the economically valuable fish products globally and shrimp farming is a fast growing enterprise in several countries. Bacterial infections account for severe mortality in shrimp aquaculture and recently emerging AHPND is reported to be caused by a plasmid bearing bacterial pathogen. Vibriosis in general has become an important cause of production loss in shrimp aquaculture. The aim of the study was to compare the species diversity, plasmid profiles and other characteristics of *Vibrio* isolates from infected and uninfected shrimp farms. Bacterial strains were isolated and identified up to species level by standard biochemical tests and API 20NE system. A total of 22 isolates were identified in which 11 strains from WSSV and EHP infected farms and 11 strains from uninfected farms. Among the isolates, *V. parahemolyticus* was prominent species followed by *V. mimicus, V. vulnificus* and *V. alginolyticus* in infected farms while, *V. fulvialis* was prominent species in uninfected farms contributing to 80%. The isolates were found to be negative for AHPND by AP4 PCR. The isolates were also tested for virulence genes such as toxR, tdh and trh. All strains were tested for the antibiotic sensitivity to 14 antibiotics and presence of plasmids. All the isolates harboured 2- 3 plasmids but the profile was different for isolates from infected and uninfected farms. The study revealed that bacterial flora is different between infected and uninfected shrimp farms. Moreover, all strains obtained from infected farms showed a common plasmid profile while those from uninfected farms were non-homogeneous.

ISAAHE-050

Genotypes of White Spot Syndrome Virus (WSSV) from Various Shrimp Farms in Kerala, India

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Outbreak due to White spot syndrome virus (WSSV) is a major problem in shrimp farming practices in India. It is highly inevitable to monitor the shrimp aquaculture system for the distribution, circulation and emergence of new genotypes of WSSV in order to prevent the severe economic loss caused by infection with the virus. In the present investigation, farmed shrimp samples (*Penaeus monodon,* n=23) positive for presence of WSSV collected from different hatcheries and farms located in Kerala, India were subjected to genotyping based on VNTR loci associated with the DNA minisatellites and the SNPs within the repeat units (RUs).The study revealed considerable variation in the genotype of several WSSV strains. The WSSV strains prevalent in the shrimp farms had 2, 4 and 6 RUs of 54 bp repeats in ORF 94 with five samples having no RU. The numbers of RUs in ORF 125, carrying 69 bp repeats were 4 and 6 with 11 virus strains carrying 4RUs. Analysis of RUs of ORF 75 with PCR indicated that 15 of the 23 samples gave a PCR amplicon of 330 bp. Ten of twenty three shrimp samples had infection with more than one WSSV strains as revealed by the presence of multiple RUs or PCR amplicons. The tested strains are closely related to Thailand and Taiwan strains of WSSV which assume that there was a migration of a putative ancestor from Thailand or Taiwan to India.

ISAAHE-051

Isopod Infestation in Freshwater Fish Farms: A Cause for Concern

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Surveillance of diseases in freshwater fish farms showed increasing occurrences of isopod infestation. The freshwater isopod *Alitropus typus* was found in five different species of hosts, viz; *Channa striatus, Wallagu attu, Catla catla*, Koi carp (*Cyprinus carpio koi*) and *Etroplus suratensis*. The isopod parasite was identified as *A. typus* based on its morphological characters. In the farm which had multispecies stocked, including Catla, Rohu, Mrigal, Common carp and Koi carp, only Catla and Koi carp were found infested with the isopod. The other fishes in the farm were free from the infestation, indicating certain amount of host specificity. Temperature and rainfall are the two important factors that affect the distribution of the parasite. The parasite was found mostly on the gills and opercular cavity of infected fishes suggesting that the parasite is highly site specific. The surface area of gills and opercular chamber is one of the important factors that determine the number of parasites infecting each fish. Skin surface and mouth cavity were the other two areas where the parasite was observed. Depending on the site of infestation, modifications in the appendages were observed. Those found on the skin of the host had long dactylus with a sharp hook which had a claw like appearance, while those on the gills had shorter dactylus with a less prominent hooked appearance. The parasites were effectively eradicated from the pond by application of quick lime raising the pH of water to 11-12.

ISAAHE-052

Effect of Immunization of Rohu *Labeo Rohita* with Inactivated Germinated Zoospores on Survival against *Aphanomyces invadans*

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Infection with *Aphanomyces invadans* is one of the most destructive diseases of freshwater fishes. Indian major carps, the major cultured species of the Indian sub-continent are highly susceptible to this disease. In the present study, inactivated germinated zoospores of *A. invadans* was used as an antigen for evaluating the effect of immunization on protection against *A. invadans* infection. In the immunization trial, 160 numbers of rohu (74 ± 12 gm) were divided into 4 groups with 40 fish each. The experimental groups were immunized intraperitoneally with inactivated (3% paraformaldehyde) germinated zoospores, inactivated germinated zoospore mixed with adjuvant (Montanide™ ISA 763 A VG) and adjuvant, separately whereas the control group was injected with PBS alone. After 21 days of first immunization, booster dose was given. After 7 days of booster dose, the fish were challenged with zoospores (1000 no./fish) of *A. invadans* and observed for a period of 4 weeks and level of protection was determined in terms of relative percent survival (RPS). There was 66% RPS in the group immunized with inactivated germinated zoospores mixed with adjuvant. Further, histopathological examination of the surviving fish indicated that the lesion area was restricted to the site of infection with well developed granulomas. On the other hand in the adjuvant control group as well as group of fish injected with inactivated germinated zoospore, although there was delayed mortality in comparison to control, all the challenged fish succumbed to infection. These preliminary findings indicated that inactivated germinated zoospores of *A. invadans* could be explored for use as one of the vaccine candidates.

ISAAHE-053

Immunomodulatory Effect of Aloin, an Active Principle of *Aloe barbadensis* Miller on an Indian Major Carp, *Labeo rohita*

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Fish are presumed to use their innate immune system as a first line of defense against microbial invasion. Being a component of innate immunity and lying at the interface between fish and the aqueous environment, skin mucus plays a frontier role in protecting the fish from infections. The stressful environment generated due to the intensive culturing of fish, suppresses their immune system, rendering the fish susceptible to infectious diseases. Several herbal products have immunostimulant properties, and are environmental friendly, economical and can act against a broad spectrum of pathogens. The present study was designed with an aim to evaluate the efficacy of aloin, an active principle of *Aloe barbadensis* on certain immune parameters in Indian major carp, Labeo rohita. Being a non-invasive method, fish skin mucus serves as an easily available source of enzymatic immune parameters. Fish were divided in three groups. First did not receive any treatment and were designated control group. Second received intraperitoneal injection of the vehicle i.e. distilled water and designated as vehicle control. Third received IP of the herbal product dissolved in distilled water at a dose of 1 mg/kg body weight for 7 days. Fishes were sampled on 2d, 4d, 6d, and 8d to elucidate the effect of aloin. The results reveal that administration of aloin elicited a significant increase in the activity of lysozyme, protease, carboxylesterase, alkaline phosphatase, acid phosphatase, as demonstrated by colorimetric and zymographic analysis. Thus, this study confirms immunostimulatory functions of aloin and hence with appropriate field trials it can be used as immunoprophylactic to prevent infectious diseases in aquaculture systems.

ISAAHE-054

The Antiprotozoal Activity of Metal Nanoparticles against Free-Living Stages of *Ichthyophthirius multifiliis*

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Ichthyophthirius multifiliis is a widespread, ciliated protozoan ectoparasite of fish. The parasite causes white spot disease, which is a major problem to aquarists, and a cause of economic distress in the aquaculture industry. In the present study, we investigated the effects of metal nanoparticles on the reproduction and infectivity of free-living stages of *I. multifiliis.* We determined that 48%, 52% and 50% of theronts could be killed within 2h of exposure to either 20 ng/mL gold, 10 ng/mL silver or 5 ng/mL zinc oxide nanoparticles, respectively. The same concentrations could kill ~50% of protomonts and tomonts after 2h exposure. Tomonts that survived showed irregular cell division and/or delayed development, with theronts released only after an extended time. Nanoparticles at concentration of 10 ng/mL silver and 5 ng/mL zinc oxide killed 100% and 97% of theronts, respectively and inhibited reproduction of tomonts after 6h exposure. Gold nanoparticles at 20 ng/mL killed 80% and 78% of tomonts and theronts 6h post exposure, respectively. *In vivo* exposure studies using rainbow trout (*Oncoryhnchus mykiss*) demonstrated that theronts, which survived nanoparticles exposure, showed reduced infectivity compared with control theronts. No mortalities were recorded in the fish groups cohabited with theronts exposed to either nanoparticles compared to 100% mortality in the control group. On the basis of the results obtained from this study, metal nanoparticles particularly silver nanoparticles hold the best promise to be used in designing and development of effective antiprotozoal agents for the management of ichthyophthiriosis in aquaculture.

ISAAHE-055

Efficacy of Commercially Available Water Probiotics on Water Quality Management in Live Feed Culture

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Health management of live feed play a significant role on survival of fish and shrimp larvae in marine hatcheries. Hence, an experimental study was undertaken on the efficacy of water probiotics (*Bacillus* and nitrifying bacteria as major strains) in improving the water quality and health of live feed. Different concentrations of water probiotics were tried for elimination of *Vibrios* in copepod and rotifer culture tanks. The study revealed a significant decrease ($p<0.05$) of *Vibrio* loads in the water and reached zero levels on the 4th day of culture in rotifer tanks. In copepod tanks, the Ammonia levels as well as the *Vibrio* loads reached zero from 3rd day onwards and remained the same till the end of the experiment. From the present study, it can be concluded that the water probiotics effectively eliminate vibrios and ammonia from live feed tanks. The present study suggested that application of water probiotics with a concentration of $1x10^4$ cfu/ml at every 10 days improve the water quality in mass culture tanks.

ISAAHE-056

Antimicrobial Activity of Hepcidin Peptide from Golden Mahseer

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AMPs are germ-line encoded short cationic peptide. Hepcidin is liver expressed multifunctional antimicrobial peptide. It is cystein rich β-sheet peptide involved in iron homeostasis and innate immunity. The cDNA contains an open reading frame of 279bp. In golden mahseer, an open reading frame encodes a pre-pro-peptide of 93 amino acids. In the present study, ClustalW alignment of nucleotide sequences of hepcidin from different teleosts demonstrated that nucleotide sequences are conserved among the teleosts. Sequence analysis revealed the presence of N-terminus sequence Q-S-H-L-S-L in mature peptide. This N-terminus sequence is crucial for antimicrobial activity against Gram (-) bacteria. In the present study mature peptide (25-mer) hepcidin was synthesized by SPPS method using Fmoc chemistry. Synthesized peptide was used to determine the minimum inhibitory concentration (MIC) by using liquid growth inhibition assay. *E. coli* DH5*α* was cultured and logarithmic phase was used for assessing antimicrobial activity of the synthesized peptide. Two-fold serial dilutions of synthetic peptide (1.5–192μM final concentrations) were prepared. Assay was performed in triplicate. 48μM concentration was revealed as MIC against Gram (-) *E. coli.* Cytotoxic assay (MTT-assay) was also performed to reveal that whether the synthesized peptide had toxic effect on host cell or not. Cytotoxic assay revealed that synthetic peptide had no cytotoxic effect on host cell. These results suggest that hepcidin from golden mahseer had antimicrobial activity and could perhaps be a better option to control bacterial infection instead of conventional antibiotics.

ISAAHE-057

Effect of Probiotics on the Shrimp *Litopenaeus vannamei* Production

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In this study, the effectiveness of probiotic application on shrimp production was evaluated. The data were collected in various shrimp farms along the east coast of India. Shrimp farms were divided into three different categories based on the application of Probiotics. Group I- probiotic application through soil, water and feed from pond preparation to harvest, Group II- application of probiotic through water and feed after stocking and continued till harvest, Group III -application of probiotic along with other supplements and chemicals. In each group 10 ponds were evaluated for the growth, survival, water quality parameters, health status and total production. All the selected farms were similar infrastructure facilities, stocking density, salinity and same season with similar culture duration. In Group I the highest shrimp growth (29±2 g), average daily growth (0.2 ±0.5 g), survival (90±2 %) and production (5.6 ± 0.2 t) was recorded in 110±5 days than the other two groups. In Group III diseases like protozoan and bacterial infections were recorded in 60 % of the shrimp farms. Further low growth rate, survival, high level of ammonia, Vibrio colonies (green and yellow) were registered in this group. Hence, the present study concluded that the application probiotic from pond preparation to harvest might enhance the survival, growth and production of shrimps *Litopenaeus vannamei*.

ISAAHE-058

Role of Probiotics in Health Improvement, Infection Control and Disease Treatment

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The growth of aquaculture as an industry has accelerated over the past decades; this has resulted in environmental damages and low productivity of various crops. The need for increased disease resistance, growth of aquatic organisms, and feed efficiency has brought about the use of probiotics in aquaculture practices.The application of probiotics in aquaculture has been widely used as a means of controlling disease, enhancing immune response, providing nutritional and enzymatic contributions to the digestion of the host, and improving water quality. Probiotics are also regarded as an environmentally friendly treatment method. The probiotics may be added to feed as live microorganisms to create a balanced indigenous micro-floral community in the gastrointestinal tract. Beneficial probiotics such as *Lactobacillus, Bifidobacterium, Streptococcus, Thermophilus, Pediococcus, Streptococcus and Carnobacterium spp. Bacillus, Flavobacterium, Cytophaga, Pseudomonas, Alteromonas, Aeromonas, Enterococcus, Nitrosomonas, Nitrobacter, and Vibrio* species are used commercially. This paper is a review of both the effects of probiotics in relation to the environment and aquaculture sustainability.

ISAAHE-059

Ontogeny of Lymphoid Organs of *Catla catla* (Hamilton, 1822)

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Ontogeny of the lymphoid organs of Indian major carp, *Catla catla* was studied histologically from hatching to 56 days post hatch (dph). The thymus was observed as undifferentiated mass of stem cells at 1 dph. The thymic tissue appeared lymphoid at 6 dph and by 12 dph thymus appeared to be developed with cortex and medulla clearly demarcated. Cortex had dense and darkly stained thymocytes where as medulla had more epithelial cells and few thymocytes. Kidney was also observed as an undifferentiated mass at 1 dph , by 3 dph, renal tubules were observed in the anterior kidney and by 6 dph, lymphocytes were clearly observed. With time course, the lymphocytes increased in number replacing the degenerating renal tubules and at 41 dph, only lymphocytes were observed in the anterior kidney. Spleen was observed for the first time at 3 dph as a small mass of darkly stained lymphopoietic cells. At 6 dph, lymphocytes were observed and with time course, the lymphocytes increased in number and at 21 dph, fully developed spleen was observed. Hence, it can be concluded that by 41 dph, all the three lymphoid organs *viz*. thymus, head kidney and spleen of *C. catla* were fully developed. The information generated in the present study may be helpful in deciding the appropriate age for vaccination to avoid immune tolerance.

ISAAHE-060

Efficacy of Oral Oxytetracycline and Potentiated Sulphonamide Therapy against *Aeromonas hydrophila* Infection in Nile Tilapia, *Oreochromis niloticus*

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Application of antibiotics for the control of bacterial diseases in aquaculture is restricted for regulatory reasons. In this study, the efficacy of medicated feeds containing approved antibiotics, viz., oxytetracycline (OTC) or potentiated sulphanamide (sulphamethoxazole–trimethoprim, SMZ-TMP) at 1g, 2g, 3g and 4g of respective antibiotics/kg feed at 2% body weight (BW) ration in preventing the *Aeromonas hydrophila* infection in *Oreochromis niloticus* was assessed. Commercial pellet feed was top dressed with respective antibiotics using 5 ml vegetable oil as binder. Fish were injected intramuscularly with *A. hydrophila* at ≈6.0×10^7 – 8.6×10^7cfu/fish and fed with OTC or SMZ-TMP feeds for 10 or 5 days, respectively. Fish mortalities were recorded during the pre-treatment, disease progression, treatment and post-treatment period. Highest mortalities (7.42 - 8.33%) were observed in challenged and untreated fish. The mortalities observed in fish fed with OTC or SMZ-TMP were 0 - 6.66% with decreasing concentrations of antibiotics from 4g to 1g/kg feed. Significant differences existed in the mortalities among fish fed with different doses of antibiotics ($P<0.05$). The relative percent survival (RPS) values were 20, 40, 40 and 60 in 1g, 2g, 3g and 4g OTC/kg feed groups, respectively; while in SMZ-TMP fed fish, the respective RPS values were 10, 100, 100, and 100. The fish fed with feed containing 2g antibiotic/kg feed at 2% BW was the lowest concentration that recorded significantly low mortality ($P<0.05$), could be the treatment of choice for the control of *A. hydrophila* in *O. niloticus* in tropical condition.

ISAAHE-061

Deciphering the Gonadal miRNAs and Transcriptome Involved in immune Response in *Clarias batrachus (magur)* (Hamilton, 1822)

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MicroRNAs (miRNAs) are small noncoding RNA molecules, which post-transcriptionally regulate the endogenous genes expression. The present work focused on identification of miRNAs and transcriptome expressed in the gonads of *C. batrachus (magur)*which are involved in the immunity response of the fish. The miRNAs transcriptome data were generated through NGS. The transcriptome data was assembled through Trinity software, which resulted in 54,061 and 1,09,204 transcripts in ovary and testis, respectively. The predicted coding sequences were annotated using BLAST. The KASS server was used to analyse KEGG pathway which revealed presence of a total of 842 unigenes that regulate the immune system. A total of 166 and 224 conserved miRNAs, along with 4 and 20 noval miRNAs, were mined from ovary and testis of *C. batrachus (magur)*, respectively, from miRNA data. Significant target genes of each conserved and noval miRNA were predicted against the 3'-UTR region of gonadal transcripts. The interaction between miRNAs and genes that regulate immunity was performed using miRanda target prediction tool, which revealed 5 miRNAs, *viz*. miR-462, miR-731, miR-146, miR-181 and miR-223,associated with the immune response in the undertaken fish. These miRNAs could be used as potential key regulator for therapeutic use to combat infection and other diseases in fishes.

ISAAHE-062

Distribution of Virulence Genes in *Aeromonas* Isolates from Diseased Fish and its Genotypic Characterization

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In this study, 42 isolates of *Aeromonas* from fish showing gross pathological lesions from different culture ponds in Kerala, India were identified on the basis of their genotypic and phenotypic characteristics as *A. hydrophila* (n=20), *A. jandaei* (n=13), *A. veronii* biovar sobria (n=3) and *A. veronii* biovar *veronii* (n=6). PCR amplification of the virulence genes such as *act*, *alt*, *ast*, *aer*A, *ahp*, *asc*V and *aop*B of the isolates revealed that the genes most frequently found in the isolates were the cytotonic heat-labile enterotoxin (*alt*) (61%) andcytotonic heat-stable enterotoxin (*ast*) (47.6%). The LD$_{50}$ value of *Aeromonas* isolates in *L. rohita* by *in vivo* challenge experiment varied from 1.5 X 10^{3}cfu/ml in *A. hydrophila* to 3.8 X 10^{6}cfu/ml in *A. veronii.* All highly virulent *A. hydrophila* isolates had *act*$^{+}$, *alt*$^{+}$, *ast*$^{+}$, *aer*A^{+}and *act*$^{+}$, *alt*$^{+}$, *ast*$^{+}$ genotype and isolates with the genotype of *act*-, *alt*-, *ast*$^{-}$, *aer*A^{-}, *ahp*$^{-}$, *asc*V^{-} and *aop*B^{-}were avirulent. Construction of phylogenetic dendrograms based on BOX-PCR banding patterns revealed that the isolates can be clustered in ten different groups and the isolates from the same source formed separate clusters. Antibiotic sensitivity assay of the isolates with 24 antibiotics revealed that 69% of the isolates of *Aeromonas* sp. were resistant to cefpodoxime, 38% were resistant to Ticarcillin and 14% were resistant to Augmentin. In conclusion, it was revealed that there is a strong correlation between isolation source, pathogenicity and genotype of the *Aeromonas* sp.

ISAAHE-063

Effect of Oral Biofilm Vaccine of *Aeromonas hydrophila* on Gut Mucosal Immunity of *Labeo rohita*

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Outbreaks of motile aeromonad septicaemia (MAS) in freshwater fish is considered to be an opportunistic pathogen and emerged as an economical burden to the rapidly growing aquaculture industry. The mucosal membrane is the gateway to systemic distribution for this pathogen. Vaccination is the most effective strategy to cope up with this disease. Mostly, aquatic vaccines are delivered by injection are considered to be the most effective one but labour intensiveness, cost and non-feasibility for small fish (large numbers) restricts its utility. Therefore, in the present study we demonstrate the immune response and efficacy of oral biofilm vaccine (BF) on gut mucosal immunity of *L. rohita. A. hydrophila* biofilm were incorporated in the feed and fed to *L. rohita* for 10 days. Admistration of BF vaccine that induces specific immune response at a local even at the systemic level. Serum and mucous samples were collected on 0, 10, 20 and 30 DPV to evaluate the immune response by *A. hydrophila* specific MAb based ELISA. Obtained results exhibited significantly higher antibody titre. Interestingly, serum and mucous giving us an idea of generation of mucosal immunity. The new approach to develop oral biofilm vaccines for aquatic diseases has great potential and essential in aquatic health management.

ISAAHE-064

Immune Response of Giant Freshwater Prawn (*Macrobrachium rosenbergii*) Fed with Brewer's Yeast Supplemented Diets

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An experiment of 75 days was conducted to evaluate the efficacy of dietary brewer's yeast, *Saccharomyces cerevisiae* on the immunostimulation, growth and survival of giant freshwater prawn, *Macrobrachium rosenbergii* and its resistance to *Aeromonas hydrophila* infection. Uniform sized prawn larvae (av. wt. 0.21 g) were stocked in FRP tanks @ 35 nos. per tank, and fed with fish meal based diet contain 0, 5, 10 and 20 g brewer's yeast /kg feed for a period of 75 days. Every 15 days growth and survival were recorded. No effect of brewer's yeast supplementation on growth and survival was observed. After the feeding trial, biochemical parameter like total haemolymph protein and immune parameters like prophenoloxidase activity, respiratory burst and total haemocyte count were measured. No significant difference was observed in mean growth, SGR and survival, but it has showed increasing trend. Immunological and cellular parameters like prophenoloxidase activity and respiratory burst and total haemocyte count were showed highest activity in 1 and 2 % brewers yeast fed prawns and they were significantly differ ($p<0.05$) from the control groups. The highest cumulative survival was observed in 1% brewer's yeast fed prawns challenged with *A. hydrophila* but increment was not significantly differ ($p<0.05$).

ISAAHE-065

Prospect of Muli Bamboo [*Melocanna baccifera* (Roxb.)]as a Phytotherapeutic in Aquaculture

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Bamboo [*Melocanna baccifera* (Roxb.)] plant plays a significant role in traditional Asian medicine and it can be used as an alternative to various chemo-therapeutics used in aquaculture. In the present study, four extracts from bamboo leaf and shoot were prepared and their phytochemical profiling was done. Study confirms the presence of various antioxidants like alkaloid, flavonoid, saponin, tannin, ascorbic acid *etc.* at various levels in allthe four extracts. Antimicrobial study reveals that the extracts are active against bacteria (*Aeromonas hydrophila, Edwardsiella tarda, Escherichia coli*) and fungi (*Saprolegnia parasitica, Aphanomyces invadans, Aspergillus oryzae*) and these tests were performed by using agar well diffusion method. Antimicrobial property of ethanol based bamboo leaf extracts (BLE) showed highest among other extracts. The toxicity study was carried on *Labeo rohita* fingerlings (average length of 10.3 ± 1.5 cm and weight 18.5 ± 1.5 g) using BLE and study showed that the maximum tolerated dose (MTD) was 20 g kg^{-1} body weight, which can be regarded as virtually non-toxic with minimal affect. Thus, it can be concluded that the BLE has the potentiality to be used as non-toxic phyto-therapeutics against bacterial and fungal pathogens in fish.

ISAAHE-066

Evaluation of Efficacy of Oxolinic Acid Against *Vibrio Parahaemolyticus* in Pacific White Shrimp, *Litopenaeus vannamei*

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In the present study, efficacy of oxolinic acid against *Vibrio parahaemolyticus* was evaluated in *Litopenaeus vannamei*. Three groups of shrimps were fed with medicated feed containing 2g, 4g and 6.65g oxolinic acid/kg feed. A dosage of 2g oxolinic acid/kg feed for 3days was found to be effective against *V. parahaemolyticus* in *L. vannamei*. Therefore, shrimp farmers may use oxolinic acid at a dose level of 2g/kg feed (15mg/kg body weight/day) for effective control of *V. parahaemolyticus.* Healthy shrimps were fed with 2 g oxolinic acid/kg of feed at the rate of 3% of body weight for elimination study. Muscle and hepatopancreas samples were collected at 1, 2, 3, 5, 7, 9, 11, 13 and 15th day after 3 days of medication period. Drug analysis was done by High Performance Liquid Chromatography. The maximum concentration (C_{max}) of oxolinic acid was found in hepatopancreas (1.99µg/g) when compared to muscle (1.04µg/g) at 16 h after cessation of medicated feeding. On the 15th day, concentration of oxolinic acid in muscle tissue and hepatopancreas found to be 0.15 µg/g and 0.12µg/g respectively. In the present study, concentration of oxolinic acid did not exceed the maximum residue level limit of 0.3 ppm in both muscle and hepatopancreas. It was found that the withdrawal time of oxolinic acid is 15 days after the cessation of medicated feeding when the residual concentrations in both tissues become less than maximum residual limit and thus safe for human consumption.

ISAAHE-067

Soapberries – Foran Eco-Friendly & Organic Method of Eradication of *Ranatra s*pp., in Carp Nursery at FSF Nandivagu Project, Telangana, India

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Ranatra species are aquatic insects which also known as long water scorpions /water stick insects, belong to the order Hemiptera, prey upon fish hatchlings and fry, also compete for food, cause major loss/damage to the spawn and fry in carp nurseries. This insect is most commonly found in our area. An experiment was conducted by giving a new intervention of an eco-friendly technique to replace the age old practice of inorganic Soap oil emulsion with complete organic members Soapberries and mustard oil emulsion to control these species during the period of June to August'2016incarp nursery of FSF Nandivagu Project, Telangana, India. Soap berries (*Sapindus*) also known as soap nuts, are native to Himalayan regions of Nepal and India were grown on a tree, has been used traditionally for hair care in the Indian Subcontinent since ancient times. This berry produces a natural soap called saponin is a natural surfactant that foams when dissolved in water, removing dirt and odours from surfaces. A thin oily film of the emulsion prepared was sprayed over the water surface to know the efficacy. Seedlings of *Labeo rohita* and *Cyprinus carpio* procured from hatchery were tested to measure the damage caused by the insect i.e. *Ranatra* Spp., for a particular period of time. The treatment was conducted with 1 control over 4 replicates. Soapberries-mustard oil emulsion is tested against the control (soap oil emulsion).The data obtained were analyzed by T-Test. An operational cost with respect to the treatment was also compared.

ISAAHE-068

Effect of Processing Treatments on the White Spot Syndrome Virus in Shrimp

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One of the most damaging viral diseases affecting the shrimp aquaculture industry is white spot disease (WSD) caused by white spot virus (WSSV), which causes high morbidity and mortality rates in shrimp and other crustaceans. To investigate the effect of processing treatments on the destruction of white spot syndrome virus (WSSV) DNA in WSSV-infected shrimps. Various processing treatments such as icing, freezing, cooking, cooking followed by slow freezing, cooking followed by quick freezing, canning, and cold storage were employed to destroy the WSSV DNA. Of the processing treatments given, cooking followed by quick freezing was efficient in destroying WSSV DNA in WSSV-infected shrimp products. Canning, and cooking followed by slow freezing process had some destructive effect on the WSSV DNA, as WSSV DNA in such processed shrimp products was detected only by nested PCR. Feasibility of frozen shrimp analysis is discussed since current viral detection is conducted in fresh shrimps before their marketing and/or re-importation. The resistance of WSSV to freezing process and cold storage based on bio-inoculation studies may alert the shrimp farmers, processors and exporters to be more vigilant on WSSV infection in farmed shrimps in order to prevent economic losses.

ISAAHE-069

Low Alkalinity and Hardness are the Predisposing Factors for the Occurrence of Bacterial and Fungal Diseases in Fish of Tripura

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Tripura is a small state situated in the north eastern region of India encompassed in between 22Â°56'N to 24Â°32'N, and 91Â°09'E to 92Â°20'E. The state is surrounded by international boundary with Bangladesh in three of sides and aquaculture is one of the major agricultural activities. Under the National Surveillance Programme for Aquatic Animal Diseases, it was reported that the occurrence of diseases like motile aeromonas septicaemia (MAS), dactylogyrosis, saprolegniasis, and epizootic ulcerative syndrome (EUS) are more as compared to other in the state. Out of these diseases, dactylogyrosis is the most reported disease followed by EUS and MAS. Furthermore, the study was conducted to evaluate the role of environmental factors in causing such diseases. The present study revealed that low alkalinity and hardness favours the development of such diseases in Tripura. However, the water pH for all the disease cases was near neutral. Hence, it can be concluded that apart from water pH other factors like alkalinity and hardness are more responsible for disease occurrence. Therefore, by maintaining proper water quality most of the disease occurrences can be reduced drastically.

ISAAHE-070

Growth and Immune Promoting Responses of Active Constituents of Chaff-Flower (*Achyranthes aspera*) Seed Extract in Larvae of *Catla Catla*

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Recently, growing interest has been paid to the immune stimulating function of medicinal herbs in aquaculture. *Achyranthes aspera* Linnaeus, a herb belonging to Family Amaranthaceae, is widely available and distributed throughout India. Among the different parts of the plant, the seed and root possess greater stimulatory activity. Efficacy of dietary supplementation of raw seed of chaff-flower (*A. aspera*) and petroleum-extract of seeds on immunological parameters of *Catla catla* larvae has been evaluated. Larvae were fed with dry diet containing raw seeds of chaff-flower, petroleum-ether extract of seeds (crude extract) while control diet was prepared without seed or extract of seed. After 44 days of feeding, fish were anaesthetized and immunized with BSA. Blood and tissue samples were collected on day 7, 14, 21 and 28 after immunization of fish. Growth (average weight and specific growth rate, SGR%), immunological parameters like total serum protein, bactericidal, myeloperoxidase (MPO), nitric oxide synthase (NOS), nitroblue tetrazolium (NBT), lysozyme activity and stress indicators (SGOT, SGPT and TBARS) of the carp were examined. The results demonstrate that the larvae fed with raw seeds and extract showed enhanced bactericidal, MPO, NOS, lysozyme activity and total tissue protein level ($p < 0.05$) as well as decreased SGOT, SGPT and TBARS compared with the control group throughout the investigation suggesting that the seeds of *A. aspera* have stimulatory effect on immunological parameters and improve the overall health status of the fish.

ISAAHE-071

Antibacterial Activity of Marine *Bacillus* Substances against Fish Pathogens and *in vivo* Evaluation Using Embryonic/Adult Zebrafish Test System

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Cell free extracts of *Bacillus thuringiensis* isolated from hydrothermal vent region of Azorean Island Faial, North Atlantic Ocean showed antagonistic against fish pathogens, *Vibrio* vulnificus and *Staphylococcus aureus*. The *in vitro* inhibition of infectious pathogens by *B. thuringiensis* was concentration dependent. The result of sensitivity to protease test showed considerable reduction of antibacterial activity for Bacillus strains, indicating the proteinaceous nature of the antibacterial substances. In addition, our results on antibacterial substance of heat resistant, proteinaceous nature and bacteriostatic mode of action was evaluated for the toxicity studies using zebrafish model, an alternative to the use of antibiotics test against infectious diseases. *B. thuringiensis* antibacterial extracts showed no toxic effects with the fish pathogens - zebra fish embryo infection studies. Furthermore, we also elucidate the use of zebrafish host model for the novel therapeutic strategies for infectious disease treatment against aquaculture pathogens.

ISAAHE-072

A New Fish Cell Line Derived from the Caudal Fin of Freshwater Oscar fish, *Astronotus ocellatus* (Agassiz, 1831): Development and Characterization

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Oscar fish, *Astronotus ocellatus* is one of the most important ornamental fish because of its interactive behavior and wide range of colours. The present study describes the development and characterization of a new cell line from caudal fin of *A. ocellatus*, designated as *Astronotus ocellatus* fin (AOF). The cell line was developed using explant method in Leibovitz's −15 medium supplemented with 20% foetal bovine serum (FBS) at 28°C. The optimum temperature and FBS concentration for cell growth were 28°C and 15%. The AOF cell line has been subcultured 44 times, since its development and the modal chromosome number (2n) was counted as 48. The AOF cell line comprised mainly of epithelial cells as confirmed by immunochemistry technique using anti-cytokeratin antibodies, an epithelial cell marker. The cell line was cryopreserved at different passage levels and the revival efficiency showed 80% viability. Partial sequence amplification and sequencing of two genes, i.e. mitochondrial 16s rRNA and COI confirmed the origin of AOF cell line from oscar fish. The cell line did not show any mycoplasma contamination as confirmed by PCR. The cells showed good transfection efficiency when transfected with pAcGFP1-N1 expression vector. The AOF cells showed cytotoxic effects in response to the extracellular products of two bacterial species, *Serratia marcescens* and *Proteus hauseri*. However, it did not show any cytopathic effects to cyprinid herpes virus-2 and viral nervous necrosis virus. Thus, the cell line can be useful for the isolation of viruses affecting oscar fishes, such as iridoviruses.

ISAAHE-073

Development of A Transgenic Cell Line for Detection of Fish Viruses

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Currently conventional methods like PCR or serological assays are employed to detect virus from suspected specimens or samples. Finally, virus isolation has to be carried out to confirm the causative agent. Such conventional methods are labour intensive and time consuming. It is quite often observed that number of negative clinical samples outweigh the positives making the task more exhausting and cumbersome. Therefore a technique which can explicitly tell the presence of any virus in a clinical sample could be quite helpful. This major research gap in diagnostic fish virology can be filled using transgenic cell line that would enable to select the precise samples for screening and virus isolation. Such a method could also permit the detection of emerging viruses that may pose a threat to aquaculture. Preliminary screening of samples for the presence of a virus using a transgenic cell line would substantially reduce both cost and effort as only positives will need to be processed further for virus isolation and characterization. It is known that viruses induce interferon which in turn stimulate the interferon stimulated genes (ISGs). Among ISGs, Mx blocks the early stage of viral replication resulting an antiviral state. Therefore, we characterized and cloned the Mx promoter of snow trout in a reporter plasmid. Here we propose that when clinical sample containing any virus is added to such transfected cell line it will produce green fluorescence indicating the presence of virus.

ISAAHE-074

Establishment of a Novel Cardiovascular Endothelial Cell Line from *Channa striatus* (Bloch, 1793) for Virological and Toxicological Studies

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An attempt was made to develop a cell line from the cardiovascular endothelial tissues of a freshwater air breathing fish (*Channa striatus*) for various applications. The vascular explants prepared from the heart were plated into fibronectin and anti-platelet endothelial cell adhesion molecule-1 antibody coated flask. After 60 h incubation, endothelial were observed in the flask. The *Channa striatus* cardiovascular endothelial (CSCVE) cell line was maintained in Leibovitz´s L-15 supplemented with 15% fetal bovine serum at 28 ºC and has been sub-cultured for more than 70 times. The authentication of the origin of this cell line from *C. striatus* was determined by PCR using primers specific to mitochondrial 16S rRNA of *C. striatus.* The cell line was further characterized by chromosome number, transfection, mycoplasma detection, cell cycle distribution, mitochondrial staining and phagocytic activity. The cells appear polygonal with a characteristic of cobblestone morphology. Expression of specific endothelial markers was observed by fluorescent staining (von Willebrand Factor, anti-platelet endothelial cell adhesion molecule-1, and anti-Endoglin) which indicates endothelial origin. Suitability of this cell line for toxicological and virological studies was tested. The cytotoxicity of ciprofloxacin on the CSCVE cell line was determined by MTT, AB, and R-123 cytotoxicity end points. Susceptibility of CSCVE cell line to nodavirus was confirmed by cytopathic effect and reverse transcriptase-polymerase chain reaction. Our studies indicate that the CSCVE cell line developed from *C. striatus* has high potential for various applications. This cell line is available in C. Abdul Hakeem College Fish Cell Line Repository for researchers.

ISAAHE-075

Establishment and Characterization of an Epithelial Cell Line from Caudal Fin of Air-Breathing Catfish, *Pangasianodon* H*ypophthalmus* (Sauvage 1978)

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Pangasianodon hypophthalmus, an air breathing catfish belonging to the family Pangasiidae, is native to Chao Phraya river in Thailand and Mekong river basins in Vietnam. It is an important fish species currently being cultured in Vietnam, Bangladesh, India, Indonesia, Malaysia, Philippines and Thailand. In the present study, a cell line has been established from caudal fin of *P. hypophthalmus* (PHF) established, using explant method. The cell line has been sub-cultured for more than 70 passages in L-15 medium supplemented with 10% fetal bovine serum (FBS). The PHF cells show optimal growth at 28°C in L-15 medium supplemented with 20% FBS. The cells have been cryopreserved in liquid nitrogen at different passages and revived successfully with 70-80% viability. Origin of cell line was confirmed by amplification and sequencing of partial fragment of mitochondrial 16S rRNA gene. The karyotype analysis revealed that PHF cells have a normal diploid chromosome number (n= 60). PHF cells showed strong positive reaction with anti-pancytokeratin antibody indicating that cell line was epithelial in nature. The cells could be successfully transfected with phrGFP II-N mammalian expression vector which indicates their utility for transgenic studies. The cells were successfully employed for cytotoxic studies as revealed by neutral red and 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. The developed cell line can be employed for virus isolation, cytotoxicity as well as transgenic studies and can be useful surrogate for whole fish studies.

ISAAHE-076

Development and Characterization of Primary Cell Culture from Testis Derived Cells of *Clarias batrachus (magur)* (Hamilton, 1822)

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The Indian catfish, *Clarias batrachus (magur) is an* important potential aquaculture species in India. In this study, an attempt was made to develop primary culture from testis derived cells of *C. batrachus (magur)*. The primary cell culture was established using explant method with different culture media compositions. Culture temperatures were examined for the proliferation of testes-dissociated cells. Culture media was also supplemented with growth factors and tissue extracts. The primary culture, developed from the fish samples collected during breeding as well as non-breeding seasons, resulted in variations with respect to cell growth pattern. The testis derived cells were able to proliferate in the media containing Leibovitz's L15 culture media with 20% fetal bovine serum at two particular temperatures, *i.e*. 25 and 28°C, up to passage no. 5. The testis derived cells were found to be epithelial in morphology and the karyotypic analysis of the *C. batrachus (magur)* cell culture showed diploid chromosome number of 50. This study will lead to establish a cell line which may be helpful in the development of an *in vitro* system for *C. batrachus (magur)* testicular cell culture and also used in various studies related to ecotoxicology as well as gonadal diseases.

ISAAHE-077

In vivo and *in vitro* Assessment of Potential Aquatic Pollutants using *Labeo rohita* model: Implications for Resources Management

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In recent time, rapid industrialization and urbanization pose serious threat to aquatic environmental pollution. Fishes respond to toxicants in a manner similar to higher vertebrates and hence serve as an appropriate model for genetic monitoring of toxic chemicals. Fishes are also chosen for evaluating toxicant loads in aquatic environments due to their sensitivity to small concentrations of genotoxic substances and their role in aquatic trophic chains. The present work was designed to determine the acute toxicity of a heavy metal compound; chromium, a pyrethroid insecticide; cypermethrin and an organophosphorus pesticide; chlorpyrifos on the fingerlings of Indian major carp, *Labeo rohita* (Hamilton, 1822) using *in vivo* and *in vitro* methods. Acute toxicity tests (96h) were conducted using a static non-renewal system, to determine the lethal toxicity of chemicals and to study fish behaviour. The data obtained from bioassay were analyzed for median lethal concentrations (LC_{50}) of the heavy metal and pesticides using Probit analysis. The LC_{50} value for chromium, cypermethrin and chlorpyrifos were found to be 765.5 ppb, 5.03 ppb and 97.05 ppb, respectively. *In vitro* toxicity of chromium, chlorpyrifos and cypermethrin were assessed based on Alamar blue and MTT assay. The IC_{50} values were found to be 70 ppb, 31.83 ppb, and 4.1 ppb, respectively. It was observed that cypermethrin is more toxic than chromium and chlorpyrifos under similar test conditions to the fish model under study.

ISAAHE-078

Modulation of Mitochondrial Morphology and MAVS Signaling by Betanodavirus *in vitro*

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Viral nervous necrosis, an infectious neuropathological disease causes mass mortality in larval and juvenile population of several teleost species and is of significant importance caused by Betanodavirus or NNV. In the present study, SISK cells displayed extensive mitochondrial fragmentation at 24hr and 48hr post infection with NNV. Quantitative real time RT-PCR analysis revealed that MAVS mRNA expression was rapidly and significantly up-regulated at 24h ($P<0.05$) and recovered to control level at 48h ($P>0.05$) *in vitro* after NNV infection. In western blot using anti zebrafish MAVS, the level of MAVS increased with time over a period of 24 h post-infection (hpi), but decreased when the NNV infection increased 48 and 72 hpi in SISK cells resulting in complete CPE. These results provide an example of host–pathogen interaction in which the virus evades innate immunity by enhancing mitochondrial fission and dislodging MAVS, a pivotal antiviral protein from the mitochondria.

ISAAHE-079

Genome Dynamics and Evolution of Codon Usage Patterns in Shrimp Viruses

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Frequent outbreaks of viral infections remain a serious threat to the global shrimp aquaculture. At present, limited information is available on virulence mechanism of shrimp viruses and their interactions with the host. We analysed the genomes and codon usage patterns of 7 small (DNA and RNA) shrimp viruses. Effective number of codon (ENC) values indicated that majority of shrimp viruses had moderate (35<ENC<50) codon usage bias. Correlation analysis between GC compositions at non-synonymous codon and synonymous codon positions (GC1,2 and GC3) as well as GC3 versus ENC curves indicated varying influences of mutational pressure on genomes and codon usage patterns. Presence of deoptimized codons and host antagonistic codon usage trends in shrimp viruses suggested the adaptation of slow replication strategy by these viruses to avoid host defences .Low CpG frequencies in all shrimp viruses as well as presence of Toll pathway in shrimp indicated that shrimp viruses have evolved with under-representation of CpGs to avoid host`s immune response. Finally, this study suggests that codon usage biases in shrimp viruses are due to inter-relationship of genome compositions, selective constraints in the form of translational efficiency and need to escape the host immune response.

ISAAHE-080

Microbial Diversity in Aquaculture Environment and Variations Associated with Next-Generation Sequencing of 16s rRNA Gene

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Next-Generation Sequencing (NGS) based 16S rRNA gene metagenomics studies are significantly contributing to understand microbial diversities in relatively unexplored agricultural sub-disciplines. However, experimental variations such as primer selection and PCR bias can have great impact on outcomes of these studies. Here, we compared two 16S rRNA variable (V3 and V4) region specific primer pairs for study of microbial diversity in sediment and fish gut samples. Clear bias in terms of diversity and community representation was observed in NGS dataset generated from V3 primers, whereas V4 primer showed much better results. Overall diversity in sediment sample was found to be much higher than gut sample. Proteobacteria followed by Verrucomicrobia were most dominant phyla in sediment and fish (*Labeo rohita*) gut samples. Other important phyla detected were Firmicutes, Acidobacteria, Chloroflexi and Tenericutes. In contrast to human and other terrestrial animals, very low abundance of Phylum Bacteroidetes and Actinobacteria was observed in sediment and fish gut samples. Presence of known cellulose degraders (*Clostridium* spp., *Ruminococcus* spp., *Eubacterium* spp. and *Bacteroides* spp.) and Short Chain Fatty Acid (SCFA) producers (*Faecalibacterium prausnitzii, Veillonella* spp. and *Megasphaera* spp.) suggested that gut microbiota played an important role in digestion, physiology, metabolism and health of *L. rohita*. Gut microbiota also harbored potentially fish and human pathogenic bacteria. Very few sequences belonging to known probiotic bacteria (*Bacillus* spp., *Streptococcus* spp. and *Bifidobacterium* spp.) were overserved. To our knowledge, this the first study from India employing next-generation 16S rRNA sequencing approach to study microbial diversity in aquaculture environment.

ISAAHE-081

De Novo Assembly and Annotation of Gill Transcriptome of *Catla catla* Using Illumina Paired-End Sequencing

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In fish, skin, gills and gut play a major role in combating infection as most of pathogens initiate the process of infection on mucous surfaces. In the present study, the transcriptome sequencing of gill of *Catla catla*, an economically important species of Indian Aquaculture, was carried out using Illumina HiScan SQ paired-end sequencing, which provided 38721751 paired-end reads. *De novo* assembly using various assemblers viz., Trinity, Bridger, BinPacker, SOAP and OASIS resulted in 91805, 37275, 26662, 41682 and 18638 contigs, respectively. Merging the post-assembly filtered contigs followed by evidential gene pipeline analysis, identified 35022 unigenes, out of which, 19605 unigenes could be annotated using Blast2GO against NCBI's non-redundant protein database. Among these, 525 genes were found to be associated with immune system. Further, some of immune genes viz. IL-1β, IL-10, MHC-I, TNFα, TGF-β, C3, iNOS, MHC-II and INF-γ were validated using qPCR, following infection with *Aphanomyces invadan*s in *C. catla*. The information generated in the present study will serve as a major genomic resource for facilitating functional genomics research in this important fish species.

ISAAHE-082

Secondary Structures of Toll-Like Receptor 3 from *Cyprinidae* family Members: A Comparative Study

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Toll-like receptor 3 (TLR3) is trans-membrane receptor protein known to recognize viral dsRNA and activate the immune responses within the host. In fish, TLR3 shows an inconsistent behavior across various species. TLR3 in fish has been reported to be stimulated on induction with virus and bacteria as well. But there is no consistency in expression of TLR3 among fish on treating with bacteria, as bacterial stimulation leads to upregulation of TLR3 in some fish species while downregulation in others. This makes it quite intriguing to reveal the structural characteristics of TLR3 among fish. So we aimed to explore the variation in properties of TLR3 among closely related fish and carried out *in silico* analysis of TLR3 from cyprinids. Bioinformatic tools were employed to find out various physicochemical properties and protein domain features of TLR3. Cyprinid TLR3 contains a signal peptide of 22 to 26 amino acids at its N-terminal followed by 15 to 18Leucine-rich repeats (LRRs). A trans-membrane region of 23 amino acid follows the LRRs which is consistent among cyprinids. Moreover, TIR-domain of 144 amino-acids is also consistent. Cyprinids demonstrated a variation in the number of LRRs while TIR, the intracellular signaling domain is similar in all species. LRRs present in ectodomain are involved in the recognition of viral dsRNA however, a variation in the length of LRRs might be a key factor that leads to altered response of TLR3 towards the same stimulant.

ISAAHE-083

Shotgun Proteomic Profiling of *Yersinia ruckeri*, the Causative Agent of Enteric Redmouth Disease in Salmonids

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Enteric redmouth disease is one of the most important diseases of salmonids. The disease is caused by *Yersinia ruckeri*, a Gram-negative rod-shaped enterobacterium. There is little information about proteomics of *Y. ruckeri.* Herein, we perform whole cell protein identification and quantification of *Y. ruckeri* strains using a label-free shotgun proteomic approach. Whole cell proteins were digested. Peptides were separated by a nano liquid chromatography system and analyzed with a high resolution mass spectrometer. The quantification of the proteins was performed using SWATH MS2 data independent technology. Differentially expressed proteins were analyzed using statistical analysis. GO annotation, subcellular localization, virulence and antibiotic resistance proteins were predicted using bioinformatics tools. The number of proteins identified in each strain was between 1193 and 1263 proteins. These included proteases, chaperones, cell division proteins, outer membrane proteins and receptors. Proteins were sublocalized in cytoplasmic, cytoplasmic membrane, periplasmic, outer membrane and extracellular. Sixteen upregulated proteins were identified in *Y. ruckeri* strains. Our study represents one of the first descriptive and comparative proteomics of *Y. ruckeri* strains. The proteins associated with virulence such as Clp, Lon, TolB, PPIases, PhoP and LuxR may be used for the construction of novel vaccines and therapeutics for yersiniosis in fish. The proteins commonly expressed such as SOD-Cu-Zn, PstS and arginine deiminase may be useful to develop single vaccination protocol and single drug therapy for both biotype 1 and biotype 2 *Y. ruckeri* strains.

ISAAHE-084

A2ML Splice Isoform Diversity in Liver Transcriptome of Indian Shad, *Tenualosa ilisha*, Revealed Using Single-Molecule Long Reads

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Alternative splicing (AS) is a major source of transcript and proteome diversity in eukaryotes. Alpha 2-Macroglobulin like1 (A2ML1), a member of innate immune system, functions as a broad-spectrum protease-binding protein. In present study, full length isoform sequencing, on PacBio RSII SMRT cells (P6C4 chemistry), was employed to characterize potential isoforms of A2ML1 gene in *Tenualosa ilisha* (common name Hilsa). An Iso-Seq cDNA library from liver total RNA sequenced generated 8.3043Gb polymerase data. Processing of raw reads resulted in 314,851 transcripts (mean read quality 94.05). Clustering and polishing resulted in 28,351 full length transcripts (>0.99 accuracy). Fifty transcripts annotated as A2ML1 were further clustered (98% similarity), resultant 14 non-redundant transcripts were analyzed for its splicing pattern, based on Homo sapiens reference (53240 bp). Total 33 exons and 8 domains were identified in consensus transcript i.e. A2M, A2M_2, A2M Comp, A2M_N, A2M_N_2, A2M_recep, Thiol-ester-cl and YfaS. Seven Hilsa A2ML1 transcripts (4736 to 1697bp) showed differential splice patterns, putatively encoding good proteins. Total 32 exons were identified in one transcript, while 23 and 19 in 2 COOH transcripts, 14, 12 and 11 in 4 partial transcripts. One transcript showed 8 domains, 2 COOH transcripts 3 and 2 , 2 partial 7 and 6, remaining two partial 5 domains each. These findings revealed a vast diversity in transcript and protein coding potential in fish genomes. Further work in this area may uncover new biological functions of AS and generate important insights into mechanisms of fish immune response to various diseases (work done under CRP-Genomics).

ISAAHE-085

In silico Prediction of Essential Genes of Fish Pathogen, *Edwardsiella tarda*

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Edwardsiella tarda, a member of Enterobacteriaceae family, is a Gram-negative, short, rod–shaped, facultative anaerobic bacterium that has been reported to cause septicaemia in a variety of cultured fish species, like catfishes, eels, IMCs etc. This bacterium is also important for its zoonotic ability. In this study, the complete proteomes of the pathogen *E. tarda* and of the host *Danio rerio* were subjected to comparative analysis to identify the proteins essential for pathogen's survival but non-homologous to the host, so that these can be used as potential drug targets. Using comparative analyses of the two proteomes, we could identify in this study 6,404 proteins in *E. tarda* that were non-homologous to the *D. rerio* proteins from about 30,000 protein dataset available in NCBI. Further, screening of these non-homologous proteins was done through Database of Essential Genes (DEG) to determine their essentiality in *E. tarda*, which revealed 449 proteins that were further analysed using Kyoto Encyclopaedia of Genes and Genomes (KEGG) database resource to predict the metabolic pathways in which they are involved. Of the identified genes from 449 proteins, majority of the genes were involved in amino acid biosynthesis and two-component regulatory system, 11 genes were found to be involved in drug resistance and 4 in bacterial secretion system. These proteins may be the potential targets for controlling the pathogen, which requires further study and characterization in the laboratory.

ISAAHE-086

In Silico Approaches for Drug Identification against *Aphanomyces invadans*

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In the present study, 81,528 protein sequences of *Danio rerio* (host) and 41,633 protein sequences of *Aphanomyces invadans* (pathogen) were taken from NCBI for *in silico* drug identification. The pathogen protein sequences having length ≤50 amino acids were filtered out and resulted in 14,223 protein sequences. These sequences were subjected to CD-HIT to obtain orthologs. Further, the proteome database of *D. rerio*, created in pearl, was blasted with the ortholog sequences of *A. invadans* at cut off at 10-4 with <30% similarity threshold that provided non-redundant protein data-set of pathogen. This data-set was subjected to the DEG using Blastp at E-value cut off at 10-4 and further analysed using KASS that provided 10 pathogen protein sequences not occurring in the host pathways. Out of 10 pathogen protein, two protein (enzyme), viz. Tryptophan synthase and Fructose-bisphosphate aldolase, were selected for inhibitor identification. The template of these two proteins (PDB ID: 1B57 and 2DH5) were obtained from PDB and modelled using EasyModeller. The PROCHECK analysis ensured the stereo chemical quality of the protein structure. These proteins were separately analysed using the BRENDA database, which provided 11 inhibitors for Tryptophan synthase and 18 inhibitors for the Fructose-bisphosphate aldolase. Thereafter, the 3D chemical structures of all the inhibitors were obtained using MarvinSketch software. These inhibitors were docked with the pathogen enzymes using AutoDock integrated with Pyrax. The inhibitors for Tryptophan synthase (5,5'-dithiobis(2-nitrobenzoate)) and for Fructose-bisphosphate aldolase (8-hydroxyquinoline) with minimum binding energy may act as potential inhibitors against *A. invadans* for developing drugs.

ISAAHE-087

Natural Genetic Diversity and Susceptibility to Diseases in Fishes

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Genetic diversity is integral to the long-term survival of a population. The populations with reduced genetic diversity might be at risk of inbreeding and result in loss of valuable alleles. This loss may result into low fertility, reduced viability and greater risk for diseases owing to reduced immune system. In other words, genetically diverse populations have greater chances to combat infections as compared to genetically homogeneous ones. The increase in infection prevalence in the wild is associated with genetic bottlenecking and inbreeding, induced by founder effects or mating systems. Over the course, habitat alteration and other man made activities have caused threats to native fish fauna, which resulted into small founder populations with low genetic diversity. In this context, it requires to document the genetic variability/divergence parameters of important fish germplasm. Individual subpopulation needs to be assayed through immune markers for knowledge on relative fitness. Four species, *Chitala chitala; Silonia silondia, Systomus sarana sarana* and *Anguilla bengalensis* are being investigated for population level discreteness using SSR markers. The species-specific novel polymorphic SSRs have been identified and validated. The intra-species diversity based on genotype data and loss of variation in genes controlling the immune system, the Major Histocompatibility Complex (MHC) will also be documented. The resultant small populations with reduced genetic diversity and low effective population size will be selected for finer analysis with genome wide markers including other immune genes. The results might have meaningful insights pertaining to scientific management of the species.

ISAAHE-088

Common Marine Ornamental Fish Diseases

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Marine ornamental fishes aptly called as “living jewels” of the ocean, have won the hearts of millions of people because of their fantastic shapes, brilliant colours and fascinating patterns. Availability of marine salts and trace elements has enabled marine aquarium fish keeping possible anywhere making it largest growing lucrative industry. Incidence of diseases by opportunistic bacteria, viruses, fungi, protozoa and parasites has increased resulting in significant economic losses. In the present study, marine aquarium shops at different regions of Chennai were visited periodically as it is one of the most important hubs for ornamental fish, in order to document the major diseases, symptoms and behavioural changes observed in the fishes maintained in aquarium setups and stocking facilities. It was noticed that the trade is dominated by clowns, damsels, butterflies and angel fishes. During the study period, several symptoms have been observed which include sluggish behaviour, spiral or erratic movement, faded and darkened pigment, exophthalmia, haemorrhages, jaw erosion, mouth and gill damage, white nodules on the gills, lesions, ulcer, furuncles, cloudy iris, cottony white growth over lens of eye and body and translucent film over the lens of eye which are the signs of diseases. Major diseases observed included bacterial diseases like fin and tail rot, viral diseases like lymphocystis, fungal diseases like exophiala infection and parasitic infestations like marine velvet, marine ich etc. Present work provides an outline of the diseases affecting marine ornamental fishes as proper intervention and precautionary measures are needed to prevent disease outbreak.

ISAAHE-089

A Review on Fish Disease Research in Assam, India

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Assam located in the North eastern region of India, has vast fisheries resources (totaling of 286,260 ha). Fish disease has emerged as an important constraint in fisheries and aquaculture in the state. Available literature suggests that Epizootic Ulcerative Syndrome (EUS) caused by *Aphanomyces invadans* (proposed as Epizootic Granulomatous Aphanomycosis or EGA in 2005) was the most frequently encountered fish disease in the state. The outbreak of EUSin India reportedly started from the Barak valley of Assam and Tripura in 1988.Saprolegniasis (by *Saprolegnia parasitica*)was less frequent/ lethal fungal disease than EUS. Most commonly encountered bacterial fish diseases of Assam were infectious abdominal dropsy (by *A. hydrophila*), followed by fin & tail rot disease (by *Pseudomonas fluorescens* and *A. hydrophila*), ulcer disease (by *Aeromonas* spp. and *Pseudomonas* spp.) and eye disease of*catla* (by *Aeromonas liquefaciens*).Argulosis (by *Argulus foliaceus*), Lernaeosis (by *Lernaea cyprinacea*), Dactylogyrosis & Gyrodactylosis (by monogenetic trematodes *Dactylogyrus* and *Gyrodactylus* sp.), Ichthyophthiriasis (by *Ichthyophthirius multifiliis*) and Trichodiniasis (by ciliate protozoan *Trichodina* sp.) were other parasitic diseases reported from Assam. Argulosis recorded the second highest incidence rate in aquaculture ponds of the state after EUS. A few cases of nutritional diseases like pin-head syndrome, lordosis and scoliosis were reported from culture ponds. Environmental diseases like asphyxiation, gas bubble disease and acidosis have also been reported from the state. The need to carry out more intensive and coordinated research covering all aspects of fish disease has been discussed in the paper.

ISAAHE-090

Experimental Fish Pharmacology: A Review

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In addition to its simplicity, water medication has the advantage that it is adaptable to mass medication of large number of fishes. Hence during drug approval for fish medication via water we should put stress on their different physiological conditions. Fish pharmacology brings together chemical evolution, implementation of quality systems and testing in aquatic animals. Fish represent the oldest and most diverse classes of vertebrates which together occupied 48% of the known member species in the subphylum vertebrate. Till now, many experiments have been done on different antimicrobial drugs and their pharmacokinetics and pharmacodynamics properties by using fish as models in pharmaceutical and toxicological research, which reveals that fish models become attractive and alternative models in that said sector. Therefore, this present article elucidates the significance and benefits of fish as a new model organism for different experimental studies on pharmacology and toxicology.

ISAAHE-091

Early Mortality Syndrome in Shrimp

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One of the most important problems in aquatic animal production especially in aquaculture activity is health and diseases management, so the fish and shrimp farmer lost several millions USD due to diseases each year. Recently, a new emerged lethal disease that termed Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Disease (AHPND) had been added to list of shrimp diseases during last recent years. This disease had caused mass mortality in China (2009) as first time and then in Vietnam (2010), afterward in Malaysia (2011) and finally in Thailand (2012). AHPND has been reported from farmed populations of the following shrimp species: *Penaeus vannamei*, *Penaeus monodon*, *Penaeus chinensis*. A bacterial isolate identified as a member of the *Vibrio harveyi* clade, most closely related to *Vibrio parahaemolyticus*, has been found to cause AHPND. Susceptible to common disinfectants. Survival to 9 and 18 days in filtered estuarine water and filtered seawater respectively. Densities of *V. parahaemolyticus* in seawater are known to be temperature dependent. Infected shrimps show gross signs including soft shells and flaccid bodies, black or darkened gills, dark edges of the pleopods and uropods, and an atrophied hepatopancreas that is whitish. Diagnosis can be done in laboratory by bacteriological tests, histology and molecular tests. Identifying the primary cause of the disease is necessary, but while this information is still not yet available, increased disease awareness and preparedness should be implemented by every shrimp producing country in the region.

ISAAHE-092

Streptococcosis in Nile tilapia: A Review

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Tilapia, *Oreochromis niloticus* is a hardy, most cultured freshwater fish in the world. It has been contributing to the world aquaculture since the ancient Egyptian days and remains a major freshwater fish species to be cultured. Streptococcosis is a disease that develops the infection by *Streptococcus* sp. They are spherical or ovoid in shape and 0.5-2.0 μm in diameter. The presence of typical clinical signs and demonstration of Gram-positive cocci from the brains, kidneys, eye or other internal organs constitutes a presumptive diagnosis of streptococcosis. Molecular diagnosis using the PCR technique is useful to identify streptococcus. Many of the PCR techniques make use of the 16S rRNA gene as the molecular marker for the identification of *Streptococcus iniae*. The presence of the pathogen in the environment of the fish is inadequate to cause a disease outbreak. Several drugs have been tested for the treatment of streptococcosis. An optimum water quality parameter should be maintained to prevent "stress'' in fish which can lead to outbreaks of disease. The diagnosis of diseased and carrier fish can be made by using rapid and accurate immunological and molecular techniques. Although chemotherapy was not really suggested, good management practice and vaccination could be parts of the plan to prevent and control streptococcosis.

ISAAHE-093

Fish Scale as an Indicator of Bacterial Infection in *Channa punctata* (Bloch, 1793)

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The study was undertaken from October, 2014 to February, 2016 to study the bacterial infection found in *Channa punctata*, a freshwater murrel collected from Deepor beel (a wetland) of Assam, India. Out of the isolated bacterial strains, isolate DJ1990 was observed to be highly virulent in artificial infection studies. The isolate DJ1990 was identified using 16S rRNA gene sequence analysis and has been deposited in GenBank with accession number KX709967.Though the skin of fish has been reported to be the most common target for infectious agents however, reports on the effect of bacterial fish pathogen on scale has not been reported so far. Damage to the epidermal layer generally provides access to infectious agents. This study deals with time and dose dependent effect of the virulent bacterial strain on fish scales. The fishes were exposed to different doses of the bacteria up to 72h of post injection with 12h time interval. Bacterial invasion and concomitant destruction in the architecture of scale along with change in pigmentation could be distinctly observed under the light and scanning electron microscopy studies. The advent of anomaly like circuli damage, lepidontal breakage, lepidontal uprooting and chromatophore dispersion etc. were found to be dependent on the pathogenic load and time of exposure.

ISAAHE-094

Molecular Characterization of Interferon Regulatory Factor-3 (IRF-3) from Indian Snow Trout, *Schizothorax richardsonii*

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A cascade of events are activated in the host celldue to multiple signaling upon recognition of viruses. As a result, viral replicationis disrupted by the induction of the innate antiviral response. Host antiviral response leads to the production of type I interferons (IFNs), a large family of multifunctional immune regulatory proteins produced by the cells resulting in an antiviral state. IFN regulatory factors IRF-3 and IRF-7 are activated by phosphorylation that regulate the expression of interferon genes upon viral infection.Intriguingly, type I IFN gene specific functions and their interactions in antiviral responses is not well studied in any Coldwater fish. Snow trout (*Schizothoraxrichardsonii*) is an important Coldwater fish inhabiting the water bodies in the foothills of the Himalayas. Using snow trout as a model, an attempt was made to understand the integrated function of type-I IFNs with the respective regulatory factors thatcoordinate and establish an antiviral state.Snow trout IRF-3 was identified and characterized byamplifying a partial fragment of 900bp. Using this partial sequence,a full length fragment of 1931 bpwas obtained byRACE.Sequence analysis of the complete IRF-3 revealed an ORF of 1371 bp along with 5' UTR of 219 bp and 3' UTR of 341 bpand,a poly A tail. IRF-3 translated region codes for a polypeptide of 457 amino acids with a predicted molecular weight of 51.372 kDa and an isoelectric point (pI) 5.10. The nucleotide sequence of snow trout IRF-3 is 84% identical with *Cyprinuscarpio*IRF-3sequence.

ISAAHE-095

Does Climate Change Aggravate Diseases in Aquatic Farming Systems? – A Case Study on Perkinsosis in Green Mussel

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Green mussel farming has gained popularity in northern Kerala but has recently witnessed unprecedented mortalities. Healthy, gaping & moribund mussels were screened for OIE listed pathogens using various diagnostic tools. Confirmatory diagnosis using molecular diagnostic techniques (PCR) followed by sequencing and bioinformatics analysis identified the pathogen as *Perkinsus olseni.* Heavy infections were observed in mussels with 100% prevalence. Resident *Paphia malabarica* also showed 100% prevalence for *P. olseni* while stray positive cases were observed in *Anadara granosa*. The year 2015-16 witnessed a sharp change in climate. Water temperatures at the culture site went up to 33°C while salinity approached 37ppt. Elevated temperature and salinities cause stress in mussels. Higher temperature and salinities are favourable for the proliferation of *P. olseni.* Stray cases of infection with *P. olseni* have been reported earlier from various wild and cultured bivalves from the region. During 2015-16 prevalence and intensity of *Perkinsus* appeared to be much higher in wild bivalves including mussels, indicating that altered climate favours its proliferation & development. The role of other resident bivalves acting as reservoirs of infection also cannot be ruled out. Farmed mussels could have acquired the infection either from these carriers or through infected, wild-collected mussel seeds used for farming or both. Though *P. olseni* has been reported in bivalves from India previously, the present study forms the first report of a *P. olseni* infection in *P. viridis* and also the first case of mass mortalities in farmed mussel in India caused by an OIE listed pathogen.

ISAAHE-096

Potential Shrimp Receptor Proteins Leading to Plausible Drug Targets Against White Spot Disease

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White Spot Disease (WSD) caused by white spot syndrome virus (WSSV), is a much devastating disease that had resulted into colossal economic losses to the shrimp farming industry in the past decades. This is highly contagious to the shrimp population and in its deadliest form it may completely wipe out the entire shrimp population within 3-7 days of the infection. Although many attempts, such as environmental control of WSSV, pre-exposure of shrimp to its pathogens, herbal treatments against WSSV, DNA/RNA-based vaccines, have been devised for effective control and management of the disease, but still no treatment is available to obstruct with the unrepressed occurrence of the disease. Thus, there is an urgent need to understand the host pathogen interaction on proteomic level between shrimp and WSSV that may provide a solution in controlling the spread of this infectious disease and ultimately save the shrimp farming industry. In one of the earlier study,Bis (2-methylheptyl) phthalate was detected as one of the suitable inhibitor among other compounds that may inhibit complex formation between host and virus proteins. Furthermore, the exact mechanisms and strategies used by this virus to infect and replicate within the susceptible host cells are also still unknown. Now, the Next Generation Sequencing technique has provided opportunities for better understanding the host defence mechanism against the pathogen. In this line, a complete documentation of the important receptor proteins of shrimp involved in proliferation of the disease can be imperative for designing plausible drug targets against the disease. The important group of shrimp receptor proteins involved in the mediation of disease are Chitin binding proteins (CBPs), Peritrophin-Like Protein (PTs), Tetraspanins, C-type lectins (CLs),β-integrins. The present paper discusses onthe important shrimp's receptor proteins and mechanisms of conciliationin the disease.

ISAAHE-097

Detection of *Flavobacterium columnare* Infection in Cultured Pacu, *Piaractus brachypomus* (Cuvier, 1818) from India.

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Flavobacterium columnare, causative agent of columnaris disease has been reported worldwide in many cultured and wild freshwater fish species and recognized as an important fish pathogen. Hitherto, there is no report of *F. columnare* infection in pacu, *Piaractus brachypomus*, from India, though the pathogen has been reported in *P. mesopotamicus* from Brazil. The present study describes detection of *F. columnare* from naturally infected pacu maintained in an experimental pond at ICAR-NBFGR (Indian Council of Agricultural Research-National Bureau of Fish Genetic Resources), Lucknow, during early winter (22 ± 2^{o}C pond water temperature). The gross lesions comprised of white patches anterior and around to dorsal fin base, tail rot and pale-yellow discolouration along with erosion of gill filaments in fish, similar to those reported for columnaris infection. Wet mount of gill tissue revealed motile bacilli under light microscope. Histologically, extensive necrotic changes and loss of architecture of gill filaments, detachment of epithelial cells and loss of cartilaginous tissue with haemorrhages were observed in adjacent filaments. Species-specific primers (FvpF1/FvpR1) produced distinct 1193 bp amplification in moribund pacu gill, while no amplification were detected in liver and kidney. Phylogenetic analysis on the basis of sequencing of 16s rDNA fragment (1193 bp), demonstrated that *F. columnare* isolate detected in pacu belongs to genomovar group II. These findings indicate *P. brachypomus* as a new host susceptible to columnaris disease.

ISAAHE- 098

Development of DNA probe for detection of *Flavobacterium columnare infection in fish*

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Columnaris disease is a bacterial infection of fish caused by the Gram-negative bacillus, *Flavobacterium columnare*. Disease outbreaks caused by *F. columnare* are increasingly in fish farms and there is a need for a reliable method to demonstrate the presence of the pathogen in the tissues. This study is aimed at developing the DNA probe for the establishment of *in-situ* hybridization (ISH) technique that could be used for the detection of *F. columnare* infection in fish. Species specific DNA probe (427bp) was designed from chondroitinase gene of *F. columnare*. The specificity of the probe sequence was validated using blastn homology search analysis. For generation of DIG labelled probes, PCR amplification was carried out using template DNA extracted from bacterial culture of *F. columnare* in the presence of DIG-dUTP supplemented dNTPs mix. DNA probes were tested for ISH on gill sections from two fish species infected with *F. columnare*; naturally infected pacu (*Piaractus brachypomus*) and experimentally infected rohu (*Labeo rohita*). Tissue sections were permeabilized and incubated with DIG-labeled probe for 12 to 16 hours at 37^0C and further incubated with anti-DIG antibody conjugated to alkaline phosphatase for 1 hour at 37^oC. Positive *in-situ* hybridization was detected using an NBT/BCIP substrate. The sections were counterstained with nuclear fast red stain. Localization of the *F. columnare* was visualized as dark brown-black deposits in the gill lamellae and gill rakers of pacu and rohu. Positive signals were absent in control (without probe) and gill sections of non-infected fish. The study demonstrates the potential use of specific DNA probe for the ISH based detection of *F. columnare* in fish tissue.

ISAAHE-099

First Record of Two Exotic Myxosporean Parasites in An Indigenous Catfish *Clarias batrachus*

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The freshwater catfish *Clarias batrachus* locally known as 'Desi magur' is an indigenous food fish of India specially known for its high content of protein, iron and other micro-elements. *Myxobolus cerebralis* is one of the most pathogenic and economically important parasite of salmons and trouts (Family-Salmonidae). It causes "Whirling" or "Black Tail" disease which afflicts fry, fingerlings and juvenile fish. The mortality rate in infected fingerlings is very high (up to 90%). During our study on parasitic fauna of freshwater fishes, we found *M. cerebralis* in brain and *M. arcticus* in kidney of *C. batrachus.* Identification of these species was based on the morphological, morphometric and molecular diagnosis. Both *M. cerebralis* and *M. arcticus* were compared to the same species reported by other workers in distant countries and other closely related species through morphometry and 18 small subunit ribosomal gene sequence analyses of mature spores. The SSu rDNA sequence data of present *M. cerebralis* (Accn. no. KM671790) displayed 99% similarity with 9 other sequence of *M. cerebralis*. The sequence analysis of present *M. arcticus* (Accn. no. KF662475) revealed that has 98% sequence similarity with *M. arcticus* from salmon of Japan (Accn. no. JN003830) and Canada (Accn. no. JN003829). This is the first report revealing two new facts; first that *M. arcticus* and *M. cerebralis* both have been isolated from a native catfish *C. batrachus* and second that *M. arcticus* is isolated from kidney (a new site of infection). The description of these two species and their inter-specific & intra-specific relationships with other *Myxobolus* species infecting different organs and hosts are discussed in the paper.

ISAAHE-100

Study on the Occurrence of Infectious Diseases in Pond Grown Indian Major Carp, *Labeo rohita in Kolleru Lake Area of Andhra Pradesh, India*

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Andhra Pradesh state predominates in polyculture of the Indian Major Carps (IMC), mostly extended between Krishna and West Godavari districts, particularly concentrated in the Kolleru lake and surrounding areas. About 0.8 million tonnes of IMC are produced annually in an estimated 1,00,000 ha in the state with 80 % share contributed by *Labeo rohita*. However, the expansion and intensification of carp culture has lead to the frequent outbreak of several bacterial and parasitic diseases in carps, resulting in loss of growth and mass mortalities. A study on the serious and unabated infectious diseases caused by bacteria and parasites in cultured rohu and their seasonal occurrence was carried out in Kolleru lake region, for a period of one year during 2014-2015. The major bacterial diseases identified were haemorrhagic septicaemia, bacterial gill disease, columnaris and abdominal dropsy. Haemorrhagic septicaemia was predominant among bacterial diseases. Among the parasitic diseases, dactylogyrosis predominated, followed by argulosis, myxosporiasis and trichidiniasis. Bacterial diseases were more prevalent in summer season, between February to July. On the contrary, parasitic diseases were more prevalent in winter season.

ISAAHE-101

Effects of curcumin supplemented diet on growth and non-specific immune parameters of *Cirrhinus mrigala* against *Edwardsiella tarda* infection

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The present research was conducted to evaluate the effect of curcumin supplement diet on non-specific immune response, growth performance and disease resistance against *Edwardsiella tarda* infection in Indian major carp (*Cirrhinus mrigala*). Fishes were fed with formulated diet containing curcumin in different graded levels as control (C) (0.00%), T1 (0.25%), T2 (0.5%), T3 (1.0%), T4 (1.5%), and T5 (2.0%) for 45 days. Treatments were maintained in triplicate and each tank was stocked with 20 fingerlings (10.5 g ± 1.4). Fishes were challenged with virulent strain of *E. tarda* after 45 days of feeding and relative percentage survival (RPS) was recorded over 14 days post challenge. The percentage weight gain, feed efficiency ratio (FER), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) of fishes fed with curcumin enriched diet were found to be significantly ($p<0.05$) better than the control group. Dietary curcumin at the level of 1.5% (T4) showed significantly ($p < 0.05$) higher RPS (62.5%) against *E. tarda* infection than control. Blood and serum samples were collected for non-specific immune parameters on 0, 15, 30, and 45 days interval and growth performance was evaluated at every 15 days period of experiment. The results suggest that fishes feed with curcumin enriched diet (1-1.5%) showed significantly ($p<0.05$) higher total immunoglobulin, serum protein, serum albumin, (Ig), respiratory burst activity (NBT) and myeloperoxidase activity (MPO). The study showed that curcumin supplemented diet @ 1.5% had stimulatory effect on non-specific immunity along with improved growth performance and increased disease resistance against *E. tarda* infection in *C. mrigala*.

Souvenir Articles

Doubling Freshwater Aquaculture Production and Sympathy to the Pathogen Cannot Go Hand in Hand: Integrated Approaches and Issues in Fish Health to Address the Concern

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Aquaculture is one of the largest growing food producing sectors around the world. Carps form the mainstay of Indian freshwater aquaculture. The three Indian major carps, *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* contribute to more than 90% of total aquaculture production. Besides three exotic carps (grass carp, silver carp and common carp), several minor carps, murrels, anabas (endmic and exotic), catfish and freshwater prawn although are being cultured on regional basis, limitation exists in their commercial farming. Research in freshwater aquaculture has evolved several epoch making technologies in India namely, polyculture of carps, integrated farming, intensive aquaculture with production capacity of more than 15.0 tonnes/ha, multiple breeding and off-season breeding, selectively bred high growth rohu 'Jayanti', quality seed production of more than twenty cultivable species, ornamental aquaculture, giant freshwater prawn breeding and culture, etc. The vibrancy of this aquaculture sector in India can be visualized by more than 17-fold growth in aquaculture that is being achieved from 0.37 MT in 1980 to more than 6.4 MT in 2014-15. It led to an unparalleled annual growth rate of more than 6% placing the country in second position in global aquaculture production. However, controlling diseases for the fish is one of the toughest challenges for the fish farmers. Addressing fish health issues has therefore become important for sustainable growth of aquaculture. Government of India along with all states has initiated the process towards doubling farm and farmers' income by 2022. Lots of debate has been underway and everyone is hopeful that some may archive the target. However, the health of fish farm and farmers and ultimate consumers would be a large concern.

There are several factors that affect the health issues of fish and contribute to their diseases and illnesses. In the past, the major cause of spread of diseases and pathogens into aquaculture systems has been mainly through movement of fish and feed and aquaculture products such as broodstock, seeds and other non-hygienic practices. The aquaculture sector has already witnessed a large number of transboundary pathogens viz., *Aphanomyces invadans* associated with EUS in 1988, *Macrobrachium rosenbergii* nodavirus causing white tail disease in freshwater prawn in 2002, Cyprinid herpesvirus 2 in goldfish during 2014-16, carp edema virus in koi carp in 2016 that has led to massive economic loss in India. In spite of increasing in number of pathogens entry to this country, the system is showing a restricted seriousness in looking or screening or surveillance of pathogens.

Doubling of production would certainly looking into approaches in both vertical and horizontal expansion of the sector including stress upon introduction of improved varieties or strains to the culture system, lots of transboundary movement and trade, integration of compatible species, intensification, feed-based aquaculture, biosecurity, disease awareness and novel diagnostics and health management approaches, etc., with limited knowledge on the carrying capacity of the environment or the system. Certainly it would led to increase occurrence of diseases, increase in use of drugs and antibiotics, emergence of drug or antibiotic resistant pathogens, entry or exist of transboundary pathogens, change in virulence of pathogens, horizontal gene transfer among the pathogens and much more incidences of secondary infections. Hence, it is necessary to develop and change in our approaches in the recent context of doubling production to handle the emergence of pathogens and the risk-associated with those. This article attempts to raise some of the health related approaches that need to be addressed during the coming future in the current context of doubling quaculture production.

Pathogen identification and characterization as top priority

The transboundary spread, emergence and re-emergence of pathogens are crucial in intensification process. Hence, it is essential to in-build disease surveillance programme in each step of intensification of aquaculture throughout the country. As a first step, the identification of pathogen forms crucial importance. Strengthening and establishing disease diagnostic laboratories in defined regions with trained human resources, development and standardization of diagnostic systems for newer or endemic organisms, looking into antimicrobial resistance pattern in both aquatic pathogens and environmental microbes, integrating ICT in health management system are some of the keys to understand pathogens. Further, characterization of pathogens using genomics or proteomics or metagenomics/metabolomics approaches are crucial for developing disease prevention and control strategies.

Integrated approaches and issues in health management

a) ***Disease surveillance at the grass root level:*** The key issue in international trade and increase in demand of aquatic products as well as increase in aquaculture production is the development and maintenance of an in-build aquatic animal health surveillance and monitoring system. Although attempt has been initiated in implementing disease surveillance in few of the states and also for few of the targeted pathogens, it needs to be institutionalized and much more strengthened involving state machineries for getting robust output form the programme. The information generated needs to well documented and must be robust interactive by which feedback need to reach to the policy makers and all the stakeholders including farmers to derive optimum benefits. It is essential to in-build risk analysis in the existing surveillance system with generation of risk based data on a farm, increasing sensitivity of diagnostics and data on variability of infection among farms. A free exchange in reliable disease information data and harmonization in reporting system will help in eradication and control of many of preventable aquatic animal diseases.

b) ***Disease zoning:*** Disease zoning is mostly used to facilitate trade and prevent further spread of any disease beyond a region. In this regard, geographical, hydrological and climatic barriers along with information on biological basis of variations in disease occurrence are crucial, and importance needs to be given on these aspects keeping in mind of our future globalization of trade of aquatic products.

c) ***Availability of information and data on each farm:*** Like shrimp farms and hatcheries, it is essential to develop policies to register all freshwater aquaculture farms and related activities under one umbrella. The GIS data along with farm or product data is essential in disease control mechanism and updating of data on a time-scale need to be there. In this regard, a mandatory mechanism needs to be in place for smooth disease control mechanism for a country like India in the context of increasing the productivity of aquaculture sector.

d) ***Farmer knowledge base:*** It is well said that people and society matter in any disease surveillance programme. Farmer-based aquatic animal disease surveillance is essential for aquaculture, particularly in a limited resource regime. Stakeholder involvement and perception of surveillance benefits, value of epidemiological data collected, farmers' knowledge, motivation and trust and institutions' functioning needs to be considered in the design of successful disease surveillance programmes. In this regard, a large number of awareness program at the farm/farmers level need to be conducted.

e) ***Metagenomics approach in epidemiology:*** The study of the distributions and determinants of the microbiome structures (community of commensal, symbiotic, and pathogenic microorganisms) and their relationship to disease is an area of recent concern in greatly understanding disease epidemiology. Metagenomics provides the possibility to explore the presence of antibiotic resistance genes in the microbes, and to identify possible 'high risk associations'. Metagenomic epidemiology will help in understanding, and eventually to predict and apply interventions aiming to limit antibiotic resistance.

f) ***Emphasis on pro- and prebiotics:*** "Care for diet" has a potential role in disease prevention in aquaculture through immune stimulation and preliminary protection in establishment of infection or disease, secondary infections in particular. In this regard, probiotics (living microorganisms for health benefit of animals) and prebiotics (indigestible fibre for benefit of probiotic organisms) have huge potential in aquaculture production and health benefits in aquaculture. It would rather a beneficial arm to get rid of antibiotics in aquaculture, and due emphasis needs to be given to this area.

g) Phytotherapy: The adverse impact of use of antibiotics, disinfectants and pesticides are being well felt. Information on a large number of herbal products are being generated, and their use in aquaculture practices needs to be streamlined.

h) Vaccine development: As on date Indian aquaculture has not come across any commercial vaccine formulations. It is high time to develop or formulate commercial formulations for important microbes involved in generating huge economic loss, that will lead to less emphasis on chemotherapeutics.

i) Selection approach in producing disease resistant varieties: Attempts have been made in developing resistant varieties of fish against aeromoniasis in India. Possibly, similar approaches need to be initiated against any other important pathogens (eg. MrNV) where vaccination or vaccine development has not been succeeded.

j) Restriction on antibiotics use and AMR: Development of antimicrobial resistance and increase in use of antibiotics are two major areas of concern world-wide. In the context of doubling aquaculture production, the farmers would rely more on use of antibiotics. Sensitization is an important step to restrict unwarranted applications of antibiotics along with providing suitable substitutes to the aquafarmers.

k) Biosecurity: Biosecurity forms an important step in the context of good management practices in a farm that has crucial role in disease entry, spread and loss related therein. Farm level biosecurity is a key area and sensitization of farmers on it is essential step in achieving success.

l) ***Trained human resources and quality aquaculture education****:* The current difficulties experienced by many countries in control of significant fish diseases is the lack of internally funded, and dedicated programmes aimed at aquatic animal diseases compared to other livestock production sectors. Such programmes will establish a skill-base that can develop experience with local growing systems and environmental conditions. These skills can then be used to tackle other diseases of importance that may emerge as aquatic production intensifies and diversifies within the region. A disease control mechanism without investing in preparedness (contingency planning, surveillance and zonation) is likely to result in expensive failure, and repeated disease outbreaks. Hence, emphasis needs to be given on trained manpower development in the sector aiming at each level of operations. All the concerned education institute under IACR, SAU, Traditional Universities and institutes under different Ministry must start action to educate the loss due to disease in fish and other crustaceans.

m) Adequate improved infrastructure and Funding support,

Political commitment, Government priorities, Farmer/industry/consumer response, Linkage on R&D, investment and infrastructure are some of the key steps in the context of increasing aquaculture production keeping aside the dreaded or emerging pathogens under control or eradication.

ICAR-CIFA's initiatives

ICAR-CIFA has taken couple of initiatives to increase fish production in the coming five years. To name a few, i) increase in number of multiplier units of 'Jayanti' rohu, an improved high yielding variety and its seed production and area of coverage; ii) selection programme on high growth catla and disease resistant rohu to aeromoniasis; iii) spreading of technologies of CIFABROOD, a brood stock diet for quality seed production; iv) seed production round the year through photothermal manipulation; v) quantity of seed production at remote places using FRP hatchery technology; vi) twenty species aquaculture; vii) selection for high growth giant freshwater prawn; viii) cryomilt for quality seed; ix) feed development for different growth stages of fish and outreach program on fish feed; x) disease surveillance in two important aquaculture states, Odisha and Andhra Pradesh; xi) molecular diagnostics for important pathogens; etc.

Within a span of three years of surveillance programme running at this Institute, it has identified two important viral disease problems in freshwater aquaculture systems viz., Cyprinid herpes virus 2 and carp edema virus, and few important secondary bacterial pathogens that are being involved in disease outbreaks as well as few new parasites.

Conclusion

There may be few other important issues that needs to be flagged off and this article is an initiative only in the context of doubling farm income keeping in mind the major bottleneck that would be played by pathogens. As it is obvious to get more and more pathogens into the system during the process of doubling the production, it is essential to apply integrated approach to get rid of those pathogens in aquaculture.

Emerging Pathogens in Brackishwater Aquaculture and Challenges in Aquatic Health Management

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Presently aquaculture is the fastest-growing food production sector, with an average annual growth rate of ~7.5%. The fisheries sector supplies 17% animal protein and supports the livelihoods of 12 % of the world's population. The global aquaculture production in 2014 recorded 74 million tonnes with an estimated value of US$160.2 billion (FAO, 2016), where India contributed about 7.3% of the global aquaculture production. The sector plays crucial role in generating foreign exchequer through export and also provides employment and livelihood to rural population. Among all the aquaculture produce, shrimp is the most valued commodity, generating valuable exchequer through export and accounts for sharing quantitatively 39.5 % with 66.1% of the total earnings from seafood export. With the introduction of exotic specific pathogen free (SPF) Pacific white shrimp, *Penaeus vannamei* in the year 2009 in India, there has been a remarkable growth in shrimp production during the past six years touching an all-time high 433,448 metric tons during 2014-2015, and export revenues crossing US$ 5 billion. Despite this phenomenal growth, the greatest threats to sustainable aquaculture are biological (infectious diseases) and chemical agents (antibiotics, agro-chemicals and organic pollutants). Aquaculture frequently suffers heavy losses due to diseases of viral, bacterial and parasitic origin that threaten the growth and sustainability of this sector. Industry-wide losses to aquatic animal diseases exceed US$6 billion per annum (World Bank, 2014). The study of ICAR-CIBA during 2006-08 in nine coastal districts covering 1142 shrimp farms indicated a loss of over 1000 crore per annum by shrimp diseases (Kalaimani et al., 2013).

Emergence of major diseases in shrimp aquaculture

Of late, it is being recognised that emerging infectious diseases have been rapidly increasing in geographical range, host range, or prevalence globally (Jones et al, 2008). Emerging infections are those that are rapidly increasing in incidence or geographic range, including previously unrecognized diseases. Trans-boundary global movements of live aquatic animals and frozen seafood products and associated rapid development of aquaculture and ecological disturbances due to climate change are often suspected as the pre-disposing factors for disease emergence and disease spread. Further, aquatic animals are displaced from their natural environments, stocked in high density, reared in artificial environments with formulated feed and water quality different from their natural ecosystem subjecting them to stress, providing increased opportunities for pathogenicity of existing infections and emergence of new pathogens (Walker and Mohan, 2009). Emerging diseases can be (i) new or previously unknown diseases; (ii) known diseases appearing for first time in a new species (expanding host range); (iii) known diseases appearing for the first time in a new location (expanding geographic range); and (iv) known diseases with a new presentation (different signs) or higher virulence due to changes in the causative agent.

The most striking emerging disease in the history of shrimp farming, the White spot disease (WSD) was first reported in June 1992 in cultured kuruma shrimp in the Fujian Province of China and in nearby Taiwan. Hoverer, the original source of emergence of WSSV in this region is not known, but it does not appear to be a natural shrimp pathogen and may have been introduced to shrimp via an unusual source of live or frozen feed. WSSV has a very broad host range of decapod crustaceans. In shrimp, the virus is known to occur commonly as a low-level persistent infection, but rapid increases in viral load, precipitated by physiological stress, salinity change or lower temperatures, can aggravate in to disease, resulting in heavy mortalities and crop loses. It is interesting to note that WSSV is the largest animal virus reported among all the animal viruses. Infectious hypodermal and haematopoietic necrosis virus (IHHNV) is a small, non-enveloped DNA virus closely related to small DNA viruses infecting *Aedes* spp. mosquitoes. It is not known if it could have jumped to penaeid shrimp. IHHNV was first discovered in *P. vannamei* and *P. stylirostris* in the Americas in the year 1981, possibly introduced along with live *P. monodon* from Asia (Thitamadee et al, 2016). IHHNV probably existed for some time in Asia without detection due to its insignificant effects on *P. monodon*, the major cultured species in Asia. The geographic variations in IHHNV isolates have suggested that the Philippines was the source of the original infection in Hawaii, which subsequently spread to most shrimp farming areas of Latin America. Yellow head disease (YHD) was the first major viral disease that caused extensive losses to black tiger shrimp farms in Thailand during 1990-91. Since then, YHD has been reported in China, Taipei, Indonesia, Malaysia, Philippines, Sri Lanka, Thailand and Vietnam. Some genomic variants other than YHV have also been reported in India. Taura syndrome first emerged in farmed Pacific white shrimp near the mouth of the Taura River in Ecuador in June 1992. The rapid spread of the virus has been attributed to international trade in postlarvae and broodstock. The original source of TSV in Ecuador is unknown, but its apparent absence prior to 1992 and the radiant nature of its spread indicate that it is unlikely to be a natural infection of shrimp. Taura syndrome virus may well have crossed species from another invertebrate host in the farming environment. Infectious myonecrosis virus (IMNV) was first detected in Brazil during 2004 in *P. vannamei* and then in Indonesia in 2006. To date, IMNV has been detected in East Java, Bali, and West Nusa Tenggara provinces. Emergence and spread of IMNV is likely to be due to trans-boundary movement of live and frozen shrimp for culture and trade.

A recent disease of farmed penaeid shrimp, commonly referred to as 'early mortality syndrome' (EMS) or technically known as 'acute hepatopancreatic necrosis disease' (AHPND), has been severely impacting productions since 2010 in southern China, Vietnam, Thailand, and Malaysia. The causative agent of EMS/AHPND has been reported to be a bacterium, belonging to the Harveyi clade, *Vibrio parahaemolyticus*. However several recent studies have indicated that AHPND pathology could be caused by several vibrio species. Virulence genes are spread among Vibrio via horizontal gene transfer. Plasmids or temperate bacteriophages transfer genetic material from bacteria to bacteria, leading to a virulence switch and the production of toxins. Vibrios are natural microflora in coastal and marine ecosystems and have only been recognized as opportunistic pathogens. However, the distinction between primary and secondary or opportunistic pathogens is artificial. The reasons for emergence of such natural microflora, which thus far remained benign and suddenly emerging as devastating pathogens is highly intriguing.

Low level prevalence of microsporidian infections by *Enterocytozoon hepatopenaeii* (EHP) associated with 'slow growth' syndromes in tiger shrimp was described in the year 2009. However, with increasing intensive shrimp farming with imported SPF *P. vannamei* in Asia, this parasite has jumped to *P. vannamei* also, causing stunted growth issues, affecting farm productions. Phylogenetically, EHP is placed within the Enterocytozoon clade, closest to the human gut pathogen *E. bieneusi* and to another intranuclear pathogen, *Enterospora canceri* that infects the hepatopancreas of the European edible crab (*Cancer pagurus*). Aquatic hosts support almost half the known microsporidian genera. The microsporidian epizootics have also been historically described to be associated with the collapse of commercial fisheries. Aquatic arthropods in particular, hosting over 50 known genera, appear to be the most affected by microsporidiosis. It is likely that sub-optimal environmental conditions and reduced immunity encourage microsporidiosis in aquaculture settings. Definitive confirmation of emergence or increased prevalence of microsporidiosis is difficult to establish for wild populations and now in farmed populations of shrimp in absence of long-term monitoring programs.

Factors contributing to disease emergence in aquaculture

For potentially emerging agents, sufficient data on their prior absence are often lacking or not documented. Improved surveillance, particularly for neglected or unsuspected hosts, geographical regions and infectious agents, would enable more effective management when new pathogens emerge. Exposure to domestic sources of infection or anthropogenic exposure to wild sources can also contribute to emergence across different hosts (Tompkins et. al., 2015). If we need to understand the emergence of diseases in aquaculture ecosystems, ecological origins of novel pathogens, genomic evolvability and its origin need to be understood. Ecological origins can be understood by undertaking environmental metagenomic studies, while the genomic evolution could be hopefully understood with next gen sequencing tools (Institute of Medicine, 2009).

Further, according to Walker and Mohan (2009), the extent of disease spread and impacts are often exacerbated by other problems such as (i) non-availability of diagnostic tools for diagnosis of the disease or identification of the causative agent resulting in infected animals going undetected (ii) poor knowledge of the potential host range (iii) inadequate knowledge of the geographic distribution (iv) no understanding of critical epidemiological factors (replication cycle, mode of transmission, reservoirs, vectors, stability), and (v) poor understanding of differences among strains. These reasons necessitate that an active surveillance programme, supported by expertise and all levels of diagnostics from classical to new generation be in place, if we have to understand and prevent spread of diseases and minimise their impacts.

Concluding remarks

To conclude, there seems to be is a classic interplay of microbial virulence and infection pressure, host defense and environmental influences which ultimately decide the outcome. Rapid growth and intensification of aquaculture also appears to be one of the major sources of anthropogenic effects contributing to disease emergence. To develop a valid strategy to predict the emerging disease will require a fusion of evolution, ecology, virology, and microbiology. Climate change and other biome-level stressors, e.g., ocean acidification, intensification of farming are likely to impart greater disease burden. Surveillance of pathogens should continue in aquaculture settings and basic studies using new generation tools such as metagenomic must be taken up in different fauna, flora and the aquatic ecosystems to build data on potentially emerging infectious agents.

Further Reading

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Perspectives in Marine Aquatic Resources and Health Management

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Aquatic resources are now regarded as major contributors to provisioning services that include health-food supply and pharmaceutical products. There are tremendous opportunities to advantageously make use of the rich biodiversity; and the provisioning, regulatory, cultural and supporting services of our seas to meet the emerging demands of humankind. Since biodiversity and ecosystem functioning is inextricably linked to human societies, we have to value the services of both, considering the growing costs of biodiversity loss and ecosystem degradation. A greener environment with enhanced ecosystem services will be beneficial to the ecological and human well-being.

Ecosystem Approach to Fisheries Management (EAFM)

It has now been recognized globally that fish and fisheries are only a part of the marine ecosystem which provides us with innumerable goods and services. This paved the way for "Ecosystem Approach to Fisheries Management" which aims at development and management of fisheries while considering the health of the marine ecosystem. India too needs to shift from traditional single species management approach to EAFM for sustainable ecosystems. EAFM addressing ecological and human wellbeing with good governance has proved to be an effective option for sustaining the fisheries in several cases. EAFM involves identifying and prioritizing issues and threats, developing and implementing plans with quality checks and adapting. An EAFM approach would address our concerns regarding production from over-burdened coastal stocks, production from under-utilized off-shore and non-conventional resources, pollution of the seas, overcapitalization of coastal fisheries, biodiversity loss and so on.

An important component of EAFM is regulated and well-managed fisheries. For well –managed fisheries we need an effective management regime that sustains the fish stock while taking care of stakeholder and environmental requirements. Just like any system, an effective management regime will have an input, processing, output and feedback components. The input is the database on fisheries statistics which needs to be strengthened. The processing component is the scientific assessment of fish stocks and ecosystems resulting in advisories on fishery regulations (spatial and temporal controls, gear controls, catch controls) in conjunction with other uses of the ecosystem (mariculture, tourism, biodiversity conservation).The output component is effective implementation of fishery regulations which is the weakest link in our current management regime. The performance of management interventions would be reviewed from time-to-time and adapted accordingly, which would form the feedback component.

The inherent strength of EAFM is that it considers the entire gamut of services provided by marine ecosystems. To effectively know the worth of a marine ecosystem, we need to value its services, both tangible and intangible. So far no work has been carried out in India on marine ecosystem valuation. A move towards EAFM gives us a seminal opportunity to work towards ecosystem valuation and to assess how various management interventions will affect ecosystem economics.

Ocean Health Index

It is common knowledge now that the oceans provide innumerable goods and services that are essential for human and terrestrial health. Thus efforts are now being made to study and understand the ocean as a whole taking into account all its tangible and intangible assets. However, this is not an easy task and we need good tools to assess the health of the oceans. The Ocean Health Index is the first assessment tool that scientifically measures key elements from all dimensions of the ocean's health — biological, physical, economic and social — to assess how sustainably people are using the ocean. This OH Index allows us to not only understand the oceans but also to prepare suitable strategies for improving the health of oceans. The OH Index deals with measurements of food provisioning services, traditional fishing opportunities, non-food products, carbon sequestration, livelihoods and economies of coastal communities, tourism and recreation value, aesthetic value and biodiversity assessment of marine ecosystems

Application of biotechnology in mariculture

The potential applications of biotechnology in mariculture include induced breeding, cryopreservation of gametes and gene banking, marker assisted genetic improvement, chromosome manipulations (especially polyploidy, in bivalves), health management like development of diagnostic, vaccines and probiotics. Functional genomics studies such as nutrigenomics offers better understanding of feed utilisation, effect of feed ingredients on growth, immunity, reproduction etc. and offers a tool for better feed formulation. The area of modern biotechnology which will probably have the most significant impact on genetic improvement of aquacultured species is transgenesis. Transgenesis offers an excellent opportunity for modifying or improving the traits like growth and efficiency of food conversion, resistance to pathogens, tolerance to environmental variables, commercially significant flesh characteristics, colour variants of ornamental species, control of reproductive activity and also in producing novel biopharmaceuticals. We will also be utilizing the microbial biodiversity of the oceans via bio-prospecting for bioactive compounds and bio-molecules..

Disease problems in mariculture and their mitigation

Disease outbreaks are the main setback in the development of mariculture programmes. Diseases caused by viral, bacterial and parasitic pathogens result in serious economic losses to the industry.

In India, the most common bacterial disease encountered in mariculture practices is Vibriosis which causes severe economic loss both in hatchery and grow-out farming. Vibriosis is the disease caused by any of the several species of Vibrio like *V. alginolyticus, V. harveyi, V. anguillarum*, etc which are the normal inhabitants of marine ecosystem. Stress induced by husbandry practices like high stocking density, increased water temperature, etc predispose the fish to infection. Similarly, disease caused by *Photobacterium damselae* ssp. *damselae* which also inhabits the marine environment causes disease when water temperature raises. Due to the limitations in use of antibiotics due to environmental concerns especially in cage or pond farming, more focus is being shed on development of alternate therapeutics and vaccines.

Among the viruses, viral nervous necrosis or viral encephalopathy and myopathy caused by betanodavirus is a serious concern especially in young ones. This virus infects most of the farmed fishes causing severe mortality. The economic loss due to the virus is chiefly due to mortality and spread of infection both vertically and horizontally.

Parasites affect the profitability of the farm by causing mortality and growth retardation. Among the parasites of farmed marine fin fish, acanthocephalan and monogenean flukes like *Gyrodactylus* and *Pseudorhabdosynochus* are noteworthy.

Development and practice of proper management system is a must in any farming venture. Stocking of healthy, disease free animals and adoption of prophylactic methods is a must for sustainable aquaculture. Research aimed at understanding the innate and acquired immune system of fish and host- pathogen interactions should merit importance to develop strategies for prophylaxis, therapeutics and metaphylaxis. CMFRI is working on development of various molecular diagnostic tools for detecting several bacterial and viral pathogens as well as parasites of maricultured species. A highly specific and sensitive RT-LAMP (Reverse Transcriptase-Loop Mediated Isothermal Amplification) technique has been developed for detecting betanodavirus in infected fish. Bivalve farming in marine and esturine environment has proved to be a sustainable farming system in India. Bivalve molluscs are filter feeders and do not require any external feed inputs, thereby minimizing the major input costs as well as the environmental impacts. Estuaries and backwaters serve as ideal locations for bivalve farming. Recently, an internationally notified pathogen, *Perkinsus olseni* has been reported from farmed and wild bivalves. Changes in the climatic pattern – rise in temperature increases the pathogenicity of *P. olseni*, causing 60-80% mortalities in farmed mussels in the northern region of Kerala. Role of increased aquaculture activities, translocation of infected stock/seed for consumption/trade could spread the disease easily. Since the farming is practiced in open waters, there is little scope for containment/treatment of the disease. Adopting better management practices, self-imposed restrictions on culture density/frequency, screening of seeds for pathogens prior to stocking and development of early diagnostic systems are advised to resolve the disease issues and reduce crop losses. LAMP based diagnostic has also been developed for detecting *Perkinsus* Sp. infection in bivalves and also for detecting WSSV in crustaceans. A nested PCR assay for the detection of Perkinsus infestation in bivalves. Profiling study on the Indian edible oyster *Crassostrea madrasensis* for the presence of *Perkinsus spp* revealed the presence of *P. beihaiensis* for the first time in *C. madrasensis* populations from the Indian subcontinent and south Asia. Development of DNA based as well as recombinant based vaccines against Betanodavirus and vibriosis is also progressing in the division. Betanodavirus antigen as well as various vibrio antigens have been cloned, recombinantly expressed and purified for development of subunit vaccines and also DNA vaccine constructs prepared using the same antigenic genes for developing recombinant as well as DNA based vaccine against viral nervous necrosis and vibriosis in fish. Various vibrio antigens have been cloned, recombinantly expressed and purified for development of subunit vaccines against vibriosis in fish. Genes for tolerance against fluctuations in temperature, salinity and acidity have been isolated from various stress tolerant microalgae using suppression subtractive hybridization technique. The isolated genes have been functionally validated by recombinant expression in *E.coli*. Among the mined genes are some potential candidates for developing transgenic stress tolerant plants.

Adaptation to climate change impacts

Climate change is now recognized as one of the greatest long-term threats to marine ecosystems and fisheries. The implications of climate change are far reaching and hence there is a need to develop and implement management actions to increase the resilience of marine systems. The action plan should comprise of the following key elements:

(i) Targeted science: Address critical knowledge gaps about climate change impacts; identify thresholds, improve monitoring, and evaluate strategies; and translate information into active management responses;

(ii) A resilient coastal ecosystem: Minimize impacts through local management actions; adapt existing management to incorporate climate change considerations; and maximize resilience by protecting vulnerable ecosystems and species;

(iii) Adaptation of industries and communities: Identify risks and resilience of fisheries industries and communities; maximize resilience by planning regulations, policies and guidelines and assist in adaptation responses;

(iv) Reduced climate footprints: Increase knowledge and involvement of stakeholders; and work with organizations and individuals to reduce their climate footprint.

Blue carbon: A win-win situation for India

Carbon dioxide is a greenhouse gas emitted from anthropogenic and natural sources, which is now modifying the atmosphere and terrestrial and ocean systems. Increasingly, carbon will be regulated and priced under national (e.g. emissions trading schemes) and international agreements (*e.g.*, Copenhagen Agreements). One of the primary natural processes that reduces carbon levels is 'Blue Carbon'. The term 'Blue Carbon' describes the natural processes by which atmospheric carbon is captured and stored (sequestered) by marine environments. 'Carbon sequestration' means carbon storage that is unlikely to be reintroduced to the atmosphere for more than some period of time (say 100 years). Coastal wetlands have the potential to sequester carbon in the tissues of plants and sediments, just as trees on land sequester carbon. Carbon sequestration and storage in seagrass, mangrove and wetland ecosystems is considered to be extremely high (rates of up to 5 times those of tropical forests) and turnover is low in undisturbed systems. And yet these ecosystems are the ones facing the greatest challenge from humans. India is blessed with large areas of mangroves and coastal wetlands which give us a distinct advantage in a carbon-led economy. However in order to utilize the economic benefits arising from 'Blue Carbon' we need to conserve these sensitive ecosystems and propagate them in the years to come. Additionally a viable market needs to be created for carbon trading (as on land – called the Green Economy), although significant efforts are required to develop this into reality, including science background and policy reform.

Better Management Practices (BMP) and Ecosystem Approach to

Aquaculture (EAA)

Developing bio-secure brood bank for high value finfish breeding and seed production programmes deserves prime attention. The broodstock tanks with continuous bio-filtration system can be used to develop and maintain brood stocks of high value marine finfish like cobia, pompano, groupers, snappers, breams, etc. Establishment of a few marine finfish broodbank is needed to provide fertilized eggs/newly hatched larvae to the hatcheries where further rearing and seed production can be carried out. A recirculation aquaculture system with components such as drum filter, fluidized-bed bioreactor, protein skimmer, UV sterilizer and egg collection facility, is inevitable for healthy maintenance of the marine finfish broodstock. The system will serve to develop the broodstock into spawners through photo-thermal conditioning. Thus the safety of the spawners and year-round controlled spawning are ensured in this system. Hence, recirculation units have to be established to ensure year-round seed production of the required species. The application of recirculation aquaculture technology for growing fish in high densities under controlled conditions is a future opportunity. This approach to fish production minimizes the use of water and land and can be potentially expanded to locations that are normally unsuitable for seafood production. The development of cost-effective grow-out feeds and appropriate health management practices also require prime attention.

Another immense opportunity for India is to augment marine ornamental fish production. On a global level, marine ornamental fish trade has emerged as a multi-million dollar enterprise. There is scope to develop breeding and seed production technologies for a number of species which have high market demand, and develop trade for hatchery produced marine ornamentals in India. The techniques for breeding more than a dozen species of ornamental fishes have already been developed by CMFRI and research focus is needed to develop technologies for more species since the trade is based on diverse species. Parallel to this, establishment of small-scale ornamental fish hatcheries can lead to income generation for rural communities. By formulating appropriate policy regulations and guidelines for wild collection of species as exemplified by the international agencies like the Global Marine Aquarium Database (GMAD) and Marine Aquarium Council (MAC), and developing commercial production of selected species through available hatchery technologies, India has the capacity to emerge as one of the major source countries for marine ornamental fish trade.

For sustainable mariculture in the country, the mariculture practices should aim at optimum production and maintain a 'green environment'. The lessons learnt from the shrimp farming is an eye opener as intensive shrimp farming resulted in environmental deterioration and consequent disease problems which called for a need for 'Better Management Practices' and species diversification. A green environment necessitates the need to adopt Ecosystem Approach to Aquaculture (EAA) by taking into account the knowledge and uncertainties of biotic, abiotic and human components of the ecosystem including their interactions, within the ecologically and operationally meaningful boundaries. In many areas, there is lack of diagnostic support for mariculture. The farmers should be educated on the negative environmental impacts that will in turn affect their production. In this regard, establishment of SPR and SPF brood facilities can go a long way to avoid the environmental health hazards to farmed fish species. An SPF certification is therefore essential. Finally, carrying capacity assessments are essential before any species is farmed either in the sea or land. This is particularly relevant while expanding sea cage farming in the country. The total number of cages in a given area, stocking density of fish per cage, and feeding intensities should be taken into consideration.

OIE-listed Aquatic Animal Pathogens and Seafood Trade Implications

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Fish is the major source of nutritional with high value protein, fats, vitamins and minerals. Fisheries provides huge employment opportunities and economic growth to the Nation. India contributes magnanimously in both marine and inland fisheries. Its potential in the field of production of fish and fishery products and export is expected to increase. India stands second globally in aquaculture production and plays an important role in the seafood trade. India's share in the seafood exports during the last decades has significantly increased over to 0.8 million tonnes and it is expected to increase tremendously at the pace of 15% this fiscal year. Seafood products are being exported to over 100 countries and the major importing countries of seafood products from India are South East Asian countries, European Union (EU), USA, Japan, China, Middle East and other countries less than 7.0%. From India over 500 seafood processing plants were involved actively in this production chain placed in East to West coast of the country. Fish and fishery products are being imported to India from other countries too. In this context of seafood trade, understanding the diseases of OIE importance and its ability to spread transboundary to the importing or exporting countries are highly essential and inevitable.

Office International des Epizooties (OIE) was formed through the international Agreement signed on January 25th 1924 headquartered at Paris to fight for animal (terrestrial and aquatic) diseases at global level. OIE became the Office of the World Organisation for Animal Health in 2003, its historical acronym OIE is used as such till now. It is an intergovernmental organisation responsible for improving animal health worldwide and is recognised as a reference organisation by the World Trade Organization (WTO). As of now over 180 Member Countries are part of OIE. OIE maintains their permanent relations with 71 other international and regional organisations. It has its Regional and sub-regional Offices on every continent. OIE has set up five Regional Commissions (Africa, Americas, Asia and Far East and Oceania, Europe and Middle East) to address the specific problems faced by their members in the different regions of the world. Asia, Far East and Oceania regional commissions have 36 member countries which includes India also. Aquatic Animal Health Standards Commission compiles information on diseases of amphibians, crustaceans, fish and molluscs and on methods used to control these diseases.

Notifiable OIE listed diseases of finfish are Epizootic haematopoietic necrosis, Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome), Infection with *Gyrodactylus salaris,* Infectious haematopoietic necrosis, Infection with infectious salmon anaemia virus, infection with salmonid alphavirus, Koi herpesvirus disease, Red sea bream iridoviral disease, Spring viraemia of carp, Viral haemorrhagic septicaemia, *Oncorhynchus masou* virus disease and Viral encephalopathy and retinopathy. Notifiable crustacean disease are Crayfish plague (*Aphanomyces astaci*), Infection with yellow head virus genotype 1, Infectious hypodermal and haematopoietic necrosis, Infectious myonecrosis, Necrotising hepatopancreatitis, Taura syndrome, White spot disease, White tail disease, Spherical baculovirosis (*Penaeus monodon*-type baculovirus) and Tetrahedral baculovirosis (*Baculovirus penaei*). This notifiable list of pathogens will be revisited as and when required by the OIE.

Some of the OIE listed aquatic animal pathogens are reported to tbe transboundary in nature. Transboundary aquatic animal diseases are the epidemic diseases (highly contagious or transmissible) and has the potential to spread very rapidly irrespective of national borders and cause serious socio-economic and possibly public health consequences. Before 1990's there are limitations in the trans-boundary movement of aquatic animals and their products, hence the pathogenic agents affecting aquatic animals were confined to the certain geographical regions of the continents. It is also observed that OIE has not listed any notifiable diseases during that period. The international expansion of aquaculture industry in post 1990's resulted in the spread of the diseases becoming a transboundary problem. Major cause was identified as globalised trade in broodstock, larvae and commodity products mainly form shrimp farming and to a lesser extent in finfish. To name few pathogens causing significant disease conditions in both the Americas (*Baculovirus penaei*, necrotizing hepatopancreatitis, Taura syndrome, infectious myonecrosis) and in Asia (spherical baculovirus, yellowhead disease, white spot disease) were reported as transboundary spread. The emergence of WSSV in penaeid shrimp in Asia were reported due to the feeding of imported frozen crabs to the shrimp broodstock in hatcheries. Infectious Myonecrosis (IMN) emerged in Brazil in 2003 and subsequently spread to *L. vannamei* farms operating in Indonesia by 2006 due to illegal trading of infected broodstock. In case of finfish Koiherpes virus were reported to be transboundary in nature and being documented in Asian countries, introduced to Indonesia through import of fish.

In General, these transboundary aquatic animal diseases were introduced and spread through movements of hatchery produced stocks, import of new species for aquaculture and development, transport of live animals which includes ornamental fish trade and brood stock enhancement, live food, feed for fish, trash fish, bait fish, sport fish, and through fish and fishery products.

In India, majority of notifiable OIE listed pathogens were not reported excepting EUS in finfish and WSSV, IHHNV and WTD in crustaceans. Under the Sanitary and Phytosanitary regulations of WTO regime, it is aimed at facilitating the trade regard to animal diseases and at the same time reducing the risk associated with the international movement of pathogenic organisms which may affect seriously aquaculture production system. Quarantine is an important measure for live transport of animals controlling the transboundary diseases. Livestock Importation Act, 1898 and its amendment bill on 2001 recommends the aquatic animals and its products tested for OIE listed pathogens.

Preventive and early warning strategies required for reducing the entry and establishment of the transboundary diseases are better border control, rapid diagnostic and surveillance tools. Lack of national strategies and non-compliance by those involved in the aqua industry may results in serious diseases. Building a knowledge base, increasing the capacity of biosecurity and quarantine facility, aquatic animal health testing infrastructure, regulatory frame works, partnerships, proper reporting and notifications and planned emergency response are the very important elements of containing a diseases. Continuous improvement of domesticated lines of brood stock for disease resistance is also highly essential. Closely monitoring the movement of live animals for the reducing the risk of pathogen spread. A continuous surveillance program to identify disease free zones must be in place which makes them free for the international trade.

Majority of fish and fishery products are traded internationally in frozen form, the transboundary spread of notifiable diseases generally cannot be neglected. Unpasteurized fish food reserved to use for the aquatic animal feed are considered as the most significant pathway of disease transmission in seafood trade rather than the fish food meant for human consumption. Plentiful volumes of seafood trade is happening worldwide and there is paucity in the data on possibility of spread of these pathogens through seafood trade.

Even though scant reports are available on outbreak of diseases due to import of fish and fishery products, this event is generally regarded as very rare or low risk of transmission. Uncertainty is always persist, because of continuous emergence of new pathogens and unidentified disease agents probably enter through the translocation of aquatic animals. Standards should be improved regularly to suit the need of the particular country.

In the present day context, ICAR-CIFT is the one of the institutions involved in surveillance of aquatic animal diseases in selected districts of Kerala and in particular the surveillance of imported fish and fishery products for the OIE listed pathogens. More than two hundred fish and fishery products imported to India through cochin port were tested during the last four years of surveillance. KHV and SVCV were not detected during the surveillance period in fish products. However, WSSV, IHHNV and MBV were detected in imported shrimp products. A single instance YHV was detected in the imported shrimp product.

Anti-microbial Resistance in Indian Aquaculture: An Overview

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Introduction

Antibiotics have been used for treatment and as prophylactic measures against bacterial infections in human and veterinary since long time. However, in due course of time, the occurrence of antibiotic resistance pathogenic bacteria becomes a serious threat. Antimicrobial resistance takes place when microorganisms transverse themselves in ways, medications used to cure becomes ineffective. The transfer of antibiotic resistance genes and selection for resistant bacteria can occur through a variety of mechanisms, which may not always be linked to specific antibiotic use. With every step towards use of antibiotics application in poultry, fish and other commodities have created a havoc for human health concerns globally. The use of antibiotics for agricultural and veterinary health purposes, particularly for growth enhancement, has played an important role in increased prevalence of antibiotic-resistant bacteria of human significance. However, no information is available on *vice-versa*. In 2013, the Centre for disease control and prevention (CDC), declared that the human race is now in the "post-antibiotic era," and in 2014, the World Health Organization (WHO) warned that the antibiotic resistance crisis is becoming dire. While, 15 multi drug resistance (MDR) bacteria have been declared a substantial threat to U.S. public health and national security by the IDSA and the Institute of Medicine, as well as the federal Interagency Task Force on Antimicrobial Resistance.

In aquaculture, very limited data is available on the specific types and amounts of antibacterials used. However, use of antibacterials in aquaculture facilities is widespread (Heuer et al., 2009). Antibacterials are mainly used for therapeutic purposes and as prophylactic agents in aquaculture operation (Sapkota et al., 2008; FAO, 2005). Prophylaxis is mostly practised in hatchery, the juvenile or larval stages of aquatic animal production. Prophylactic treatments are also thought to be more common in small-scale production units that cannot afford, or cannot gain access to, the advice of health care professionals. Thus, the aquatic animals are in the risk of antibiotic resistance and thereafter, due to biomagnifications the huge amount of antibiotics is getting accumulated. .

Development of antibiotic resistance

The multidrug efflux systems contribute significantly to the increased resistance to multiple antibiotics in bacteria. Metagenomics and functional genomics studies have provided compelling evidence that antibiotic resistance genes are widespread and the natural reservoirs of potential antibiotic resistance include many ecosystems such as in agriculture, the gut of humans and food animals, and even ancient soils. The diverse range of novel antibiotic resistance genes could be accessible to clinically relevant bacteria and play a critical role in the emergence of antibiotic resistance among pathogens. The presence of antibiotic resistance genes in the aquatic environment is demonstrated by Fahrenfeld et al. (2013) and Suzuki et al. (2013). In aquaculture, the method of antibiotic administration is through feed as medicated feed, immersion or directly to the feeds as a solution forms. Generally, it has become a practice to control bacterial disease outbreaks in cultured fish through the medicated feed. Medicated feeds are commercially prepared, and contain an antibiotic to control specific bacterial infections by either killing the bacteria or preventing the bacteria from reproducing. Thus, care must be taken while using in feed and precision of dosing interval which will ensure the complete treatment on effective dose without much wastage and overdose. It will save the cost on medicament and same time reduces the environmental damage and resistance. The continuous use of antibiotics in feed has been expressed a concern of developing drug resistant strains of pathogenic microorganisms. Therefore, medicated feeds should be used according to manufacturer's instructions, with particular attention to withdrawal periods. Another important concern regarding the development of resistance is that feeding of lower concentrations of antibiotics than the effective dose or decreasing the number of days the drug fed, should be strictly prohibited.

Pattern in drug resistance

- Antibiotics are recognised as the life saving drugs. However, up to 50% of the time antibiotics are used in indiscriminate fashion (incorrect dosing or duration).
- The microbes that contaminate food can become resistant because of the use of antibiotics in people and in food animals. *For instance*, the bacteria *Salmonella* sp. and *Campylobacter* sp., it is primarily the use of antibiotics in food animals that increases resistance. Because of the link the between antibiotic use in food-producing animals and the occurrence of antibiotic-resistant infections in humans, antibiotics that are medically important to treating infections in humans should be used in food-producing

animals only under veterinary oversight and only to manage and treat infectious disease, not to promote growth.

- The other major factor in the growth of antibiotic resistance is spread of the resistant strains of bacteria from person to person, or from the non-human sources in the environment. Further, bio-magnification play an important role in antibiotics resistance in top consumers of the food chain.

Antibiotic resistance *vis- a- vis* aquaculture

Aquaculture is the fastest growing food sector in the world. However, there is argument on its sustainable growth and intensification. High density dependent intensification is a major risk for disease outbreaks in aquaculture (Interact, 2010). Disease outbreaks include trans-boundary aquatic animal diseases, infections due to viruses, bacteria, fungi, parasites and other pathogens. Disease outbreaks in aquaculture, thus, are impeding both economic and social development. In India, Andhra Pradesh contributes a major share of 40,000 tonnes of major carps, worth of 600 million rupees per year, but loss due to disease outbreak is supposed to be INR 40 million. It is reported that a bacterial pathogen, *Aeromonas hydrophila* alone responsible for causing mass mortalities (30 – 70%), in Indian major carps. Antibiotics administered in fish are excreted with urine or faeces and attend the sewage treatment. The main consequences of use and abuse of antibiotics is the development and diffusion of antibiotic resistance that has become a serious global problem. To control different bacterial diseases, antibiotics are widely used in aquaculture and cause further consequences as AMR through leaching and due to less palatability in the whole aquatic system.

Leaching of drug in aquatic system

Leaching of drug into the water will occur with all kinds of medicated feeds, but is especially high with surface coated pellet feeds. The extent of leaching depends on various factors including the water solubility of the chemical, the time during which the feed remains in the water before the fish eat the pellets, and the size of the pellets (ratio of surface area to weight). The smaller the pellets the higher will be the leaching losses. Leaching is also influenced by the reduced palatability, less bioavailability, pharmacokinetics and pharmacodynamics properties and withdrawal period of the medicine.

Palatability of medicated feed

Depressed palatability is an important drawback of feeds that originates from the drugs itself, from the binders used to medicate the feed. Bearing in mind that already diseased fish have the tendency to stop feeding, this problem can be of crucial importance for the success of treatments. Several fish species, notably carps, are particularly delicate and fussy. Even fishes like *Pangasianodon hypophthalmus* sometimes hardly consume medicated feed if it is not attractive enough. It was reported that, surface coated oxytetracycline was highly repellent for sea bass and reported depressed feed consumption by 90% (Rigos et al. 1999).

Status on antibiotic resistance pattern in pathogenic bacteria of fish.

Our study on the bacterial isolates of fish origin from Andhra Pradesh and Odhisa revealed that *Pseudomonas* spp, *Aeromonas hydrophila* and *Myxobacteri*a spp. are resistant to various antibiotics which are generally used in clinical settings for human, terrestrial and aquatic animal chemotherapy. In addition, statewise important aquatic bacteria those are AMR are mentioned as belows and in Fig.1,2.3.4.5.6

***Pseudomonas* sp.**

Out of 32 antibiotics screened, *P. putida* resistant to Cephotaxime, Co-trimaxime, Ampicillin, Cephalothin, Ceftazidime, Tricarcillin and Cloxacillin. *P. aeruginosa* was sensitive to Gentamycin, Tobramycin, Netillin and intermediate sensitive to Chloramphenicol, Oxytetracycline, Tetracycline while resistant to other antibiotics. *P. fluorescens* was sensitive to Tobramycin, intermediate sensitive to Gentamycin; resistant to rest other antibiotics. Tiwari et al. (2016) reported that injudicious use of drugs is one of main contributors to emerging resistance of *P. aeruginosa* to antimicrobial therapy. It also contributes to appearance of multidrug resistant strains of bacterium.

Aeromonas hydrophila

One of our study suggested that most of the strains of *Aeromonas hydrophila* are resistant to Cephotaxine, Cephalexin, Co-trimaxozole, Ampicillin and Cefuroxime. In another study, we found that *Aeromonas hydrophila* isolated from fish at eastern India are mostly partially resistant to tetracycline (38.5%) ciprofloxacin, gentamycin and flomequin (30.8%)(Samal, 2015). It is reported earlier that *Aeromonas hydrophila* are resistant to tetracycline, streptomycin, erythromycin, cephalosporins (cephalothin, cefotaxime and cefoxitin), nalidixic acid, sulfadiazine/trimethoprim, penicillin-G, colistin, ampicillin, novobiocin, amoxicillin, oxytetracycline, amikacin, gentamicin, oxacillin, nitrofurantoin, augmentin, piperacillin, ampicillin- sublactum, cefotaxime, levofloxacin, ciprofloxacin, lincomycin, rifampicin, chloramphenicol, amoxicillin, metronidazole, neomycin, ticarcillin, mezclocillin, sulfamethoxazole, trimethoprim, vancomycin, fluoroquinolones, clindamycin, erythromycin, , polymixin-B, co-trimazole, sulphafurazole, carbenicillin, chlortetracycline, cloxacillin, doxycycline, cefaclor, norfloxacin, bacitracin, kanamycin and cefodoxime (Abbot et al., 1992)

Flavobacterium sp.

All isolates are found to be resistant to cefuroxime, cloxacillin, cephalothin, ampicillin, penicillin G and trimethoprim. Most of the isolates were found to be resistant to co-trimoxazole, bacitracin, neomycin, nitrofurazone and norfloxacin.

Vibrio sp.

All the strains are found to be resistant to cefuroxime, cloxacillin, cephalothin, ampicillin, bacitracin and neomycin. Behura (2015) studied the resistance pattern of antibiotics against 21 different antibiotics. Most of the *Vibrio* spp. isolates from fish were resistant to cefuroxime, cephalothin, ampicillin, bacitracin,, neomycin, , penicillin and cloxacillin

Staphylococcus aureus

All strains are found to be resistant to cefuroxime, cephalothiin, ampicillin, penicillin and co-trimoxazole. Most of the strains sensitive to ciprofloxacin, nalidixic acid, chloramphenicol, tetracycline and erythromycin.

Edwardsiella tarda:

Antibiogram studies indicated that the bacteria are resistant to trimethoprim, tetracycline, neomycin, gentamicin, cefuroxime, cephalothin and resistant to ampicillin, erythromycin, nalidixic acid, oxytetracycline and amicacin respectively.

Ways forward:

Focus on Pharmacokinetics and pharmacodynamics of drugs

Pharmacological research on aquaculture drugs is limited to very few antibiotics. Therefore the development and commercialisation of new medicines for treatment of aquatic diseases are scarce. The burgeoning incidences of diseases in aquaculture has lead high demand for better knowledge on medicine, art of treatment and the availability of quality pharmaceutical and hazard free biological products suitable both for the prevention and treatment. However, this sector has largely benefited from in-feed medication and the progress made by the pharmaceutical industry on modern formulations. But a lot of scope prevails specifically in area of innovative oral delivery systems, such as bio-encapsulation, nano-delivery, conjugation and smart delivery, micro-particulate diets and protected micro-diets. Another big shortfall is lack of species wise pharmacokinetic studies and most pharmacokinetic studies have been performed on only few species, most notably in salmonid fish, or other temperate species. Usually, disease prevalence are more in tropics, beside this there are considerable inter-specific variations in the pharmacokinetics of antibacterial compounds, so species-specific information is always needed before determining a suitable product to be used.

The precision of knowledge on drug absorption, distribution and elimination (metabolism and excretion) (basic pharmacokinetic studies) and the bioavailability of the active ingredient from two or more product formulations and/or routes of administration (bioequivalence studies) in different species is essentially required before making a medicated feed so efficacy cost effectiveness and safety from toxicity and environmental hazards can be maintained. Other important aspects of pharmacokinetic studies include the determination of withdrawal periods and the estimation of residues introduced into the environment. Drug dosage regimens should be host and environment dependent. Fish species reared in warm water may absorb, metabolise and excrete drugs at a different rate (often faster) than those in cold water. The salinity of the holding water also affects drug kinetics. Fish kept in saltwater drink more water than freshwater fish. Thus, antibacterial in the gastrointestinal tract of fish species held in saltwater may bind, which can reduce their uptake (Smith et al., 2008; Toutain et al., 2010). The same have been already reported for tetracycline that has low bioavailability even in freshwater. The half-lives of drugs in fish are highly dependent upon the dosage regimen, the route and the temperature. Therefore, temperature becomes a very important factor in deciding on the dose and treatment intervals (Toutain et al., 2010).

Withdrawal time

Withdrawal time for drug must be observed to ensure that drug used in a target animal does not exceed lethal tolerance levels in the animal tissue at the time the edible portion is made available for human consumption. Following proper withdrawal times helps to ensure that products reaching consumers are safe and wholesome. Withdrawal information is found on the product label, package insert, or feed tag of any approved product. Withdrawal requirements for drugs used in an extra-label manner must be determined by the prescribing licensed veterinarian. Withdrawal times are usually reported as a specific number of days. Each withdrawal day is a full 24 hours, starting from the last time an animal receives or is exposed to a regulated compound. However, very limited studies on the withdrawal time have been made for antibiotics used in aquaculture. Therefore, future focus must be given to understand the withdrawal time of the commonly used antibiotics in Indian aquculture. As far as human health is concerned the withdrawal period has to be defined properly and time and dose of antibiotics also need to be standardised.

Looking for alternatives

One of another possible solution to chemotherapies in aquaculture is related to the use of herbal extracts. Herbal extracts from plants or algae are known to contain natural compounds, such as phenolic compounds, polysaccharides, proteoglycans and flavonoids which are able to stimulate the fish immune system and therefore may play a major role in the prevention or control of infectious microbes (**Misra** et al., 2006; Rao et al., 2006; Sahu et al., 2007; Sasal, 2014).These are recognized as eco-friendly alternatives for therapeutic and prophylactic purposes in health management of aquatic animals, which combine sustainable production systems with high quality of seafood products; nevertheless, further studies are needed to assess any potential impact of these substances on the host microbiota and on the environment.

Conclusions

The reason for developing antibiotic resistance strain include over/lower use and misuse of antibiotics, scarcity of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements. Considering the rapid growth and importance of the aquaculture industry, intensive, and often unregulated use of antibacterial agents for animal production. Additional efforts are required to prevent the development and spread of antibacterial resistance in aquaculture. Governments, farmers and veterinary surgeons all have a shared responsibility to ensure that medicines are used judiciously. Further the route and carrier for antibiotic resistance may also be taken into consideration.

India should phase out antimicrobial growth promoters from livestock when these drugs are medically important and when these are premixed with feed. Such a move would have regional consequences and would send a strong signal of the country’s commitment to tackle this issue The Indian Council of Medical Research has established a National Programme on Antimicrobial Surveillance in ten laboratories and covering priority pathogens identified by the World Health Organization.. Further, presently FAO and ICAR have started initiatives towards working together to access the AMU and degree of AMR in animal and fish so that country will be coming under ONE health programme toward AMR. In the same line, some more efforts are needed to be taken in collaboration with other research institutions and organisations for developing an effective surveillance and monitoring of AMR in fisheries and aquaculture in India.

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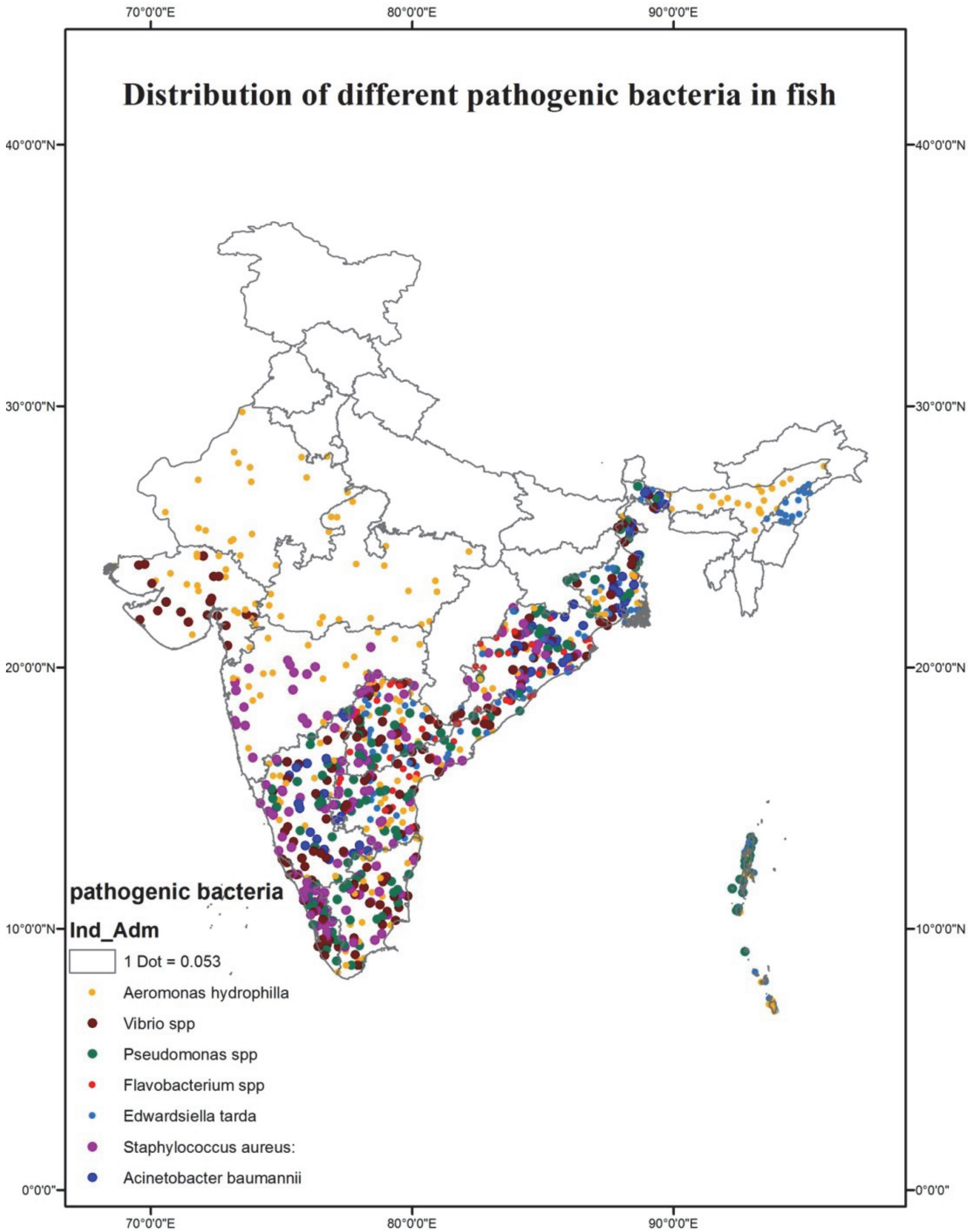

Fig.1 State-wise distribution of pathogenic bacteria in fish

Advances & Challenges in Health Management of Coldwater Fishes in India

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Disease is the single largest cause of economic losses in aquaculture inviting our concern towards proper health manage in fish culture activities with a view to increase farmer's income in coldwater sector. It is imperative to effectively address the challenges of infectious microbial agents of viral, bacterial, parasitic and fungal origin as well as non-infectious pathological conditions in coldwater fish health management. In any water body, the presence of pathogen is not alone a factor to manifest a disease outbreak. The interaction between pathogen-host-environment becomes the basis for disease occurrences. Therefore, understanding the coldwater environment, the fish species under culture and the maneuvering of farming conditions are prerequisites for effective fish health management. Along with gaining knowledge of the basis over which diseases outbreaks occur, we also need to develop our ability to detect pathogenic organisms available in the carrier fish or in the aquatic environment. In this endeavor, ICAR-Directorate of Coldwater Fisheries Research (ICAR-DCFR) in recent past has initiated compressive research programmes related to the fish health which included the ecology and epidemiology of viral, bacterial, parasitic & fungal pathogens in coldwater enironment. The present article not only envisages the documentations of viral, bacterial, parasitic & fungal diseases & their pathogens, but also provides information on recent research advances commenced by the ICAR-DCFR on coldwater fish health management.

(i) Viral Diseases in Coldwater Fishes

The important viral diseases reported from coldwater sector are infectious pancreatic necrosis (IPN), viral hemorrhagic septicaemia (VHS), spring viremia of carp (SVC), epizootic hematopoietic necrosis (EHN) & infectious hematopoietic necrosis (IHN). However, our investigations could not come across any one of such problems in the Himalayan region of our country. Data obtained after extensive survey studies in coldwater aquacultures practices of Himachal Pradesh, Jammu & Kashmir, Uttarakhand & North-East regions appeared ambiguous and signatures of virus have not be confirmed. Although viral signatures are not so far recorded from the cold water sector in India, yet effective surveillance programmes on national platforms has been carried out to keep eye on the appearance of any viral infections.

(ii) Tracking Bacterial Pathogens in Coldwater Aquaculture

Bacterial diseases and identification of their causative pathogens have been studied in depth in coldwater aquaculture. In present aquaculture scenario, the rainbow trout, *Oncorhynchus mykiss,* is established as a prime cultivable coldwater fish species and rapidly gaining prominence in our country. A compressive data sheet of pathogenic bacteria flora, their occurrences, seasonal distribution, prevalence in trout & nature of sensitivity/resistance property of isolates to commercial antibiotics applied is developed for selected trout farms in Indian Himalayan Region (IHR) under in-house research programme.

Trout farms of Uttarakhand in central Himalayan region signify the presence of Aeromonas hydrophila, Aeromonas veronii (ichthiosmia), Aeromonas popoffii, Aeromonas allosaccharophila, Pseudomonas fluorescence, Lactococcus garvieae, Citrobacter freundii, Escherichia coli, Micrococcus, Acidovorax facilis, Bacillus, Enterobacter, Brevibacillus agri, Shewanella, Morganela, Gamma proteobacterium & Hafnia alvei. The ubiquitous presence of different species under genus Aeromonas is considered as the commonly occurring opportunistic primary trout pathogens in all three major seasons; summer, monsoon and winter. The other major bacterial pathogen that have been frequently isolated along with Aeromonas hydrophila from moribund rainbow trout showing clinical symptoms of lesion in caudal peduncle region, tail, fin and gill rot is Pseudomonas fluorescence.

Analysis of microbial samples from trout farms of Sikkim in northeastern Himalayan shows presence of more or less geographically diversified bacterial population in comparison to trout farms of central Himalayas. Identified bacterial flora is dominated by population of *Serratia*, *Pseudomonas putida*, *Micrococcus*, *Vagococcus*, *Hafnia alvei, Morganella, Rahnella aquatilis, Klebsiella, Pantoea, Pseudomonas veronii, Carnobacterium maltaromaticum, Carnobacterium divergenes, Pseudomonas fluorescens, Yersinia, Erwinia & Corynebacterium sp. Carnobacterium* and *Corynebacterium* are gram positive bacteria, reported to cause pseudo kidney disease in rainbow trout and brain swelling in carp culture. Genus *Pseudomonas* & *Carnobacterium* are considered as predominant groups in trout farms of Sikkim.

Large scale establishment of trout raceways and farming in Jammu & Kashmir and Himachal Pradesh of northwestern Himalayan is done with mammoth success due to its agro-climatic condition, topography and presence of huge perennial sources of snow-fed Himalayan rivers and streams. Based on hemolytic activity, virulence and serum activity study some important bacterial pathogens have been isolated from trout farms of Jammu & Kashmir and characterized by amplification of 16s rRNA, gyrB gene & rpoD gene of *Aeromonas hydrophila, Aeromonas allosaccharophila, Aeromonas sobria, Hafnia alvei, Citrobacter freundii, Serratia sp, pseudomonas fluorescence* and

Enterobacteriaceae. Himachal Pradesh being one of the major trout producing states, considerable attention was paid to studying bacterial population and their diversity in trout farms. Isolates identified in lieu of bacteria population from different trout farms of Himachal Pradesh delineated presence of *Lactococcus garvieae*, *Citrobacter freundii, Proteus hauseri, Pantoea agglomerans, Erwinia rhaphontici, Aeromonas hydrophila, Aeromonas veronii, Hafnia alvei, Pseudomonas fluorescens, Pseudomonas geniculata, Plantibacter, Escherichia coli, Lelliottia amnigena, Microbacterium, Staphylococcus, Plegiomonas, Mollerella wisconsensis, Enterococcus casseliflavus, Enterococcus gallinarum, Caronobacterium divergens, Caronobacterium maltaromaticum, Bacillus firmus, Stenotrophomonas, Kluwea* and *Paenibacillus.* The predominance of bacteria in few trout farms of Himachal Pradesh were identified to belong to the family *Enterobacteriaceae*.

Precise data generated on bacterial germplasm of rainbow trout farms of central, northeastern and northwestern Himalayan regions give ample chance to formulate location specific health management practices in rainbow trout farming. Scientific data generated on the subject would facilitate generating thought-out standpoint controlling challenges related to pathogenic microbial infections in trout health management.

(iii) Ulceration in rainbow trout

Periodical monitoring of trout health has recorded 20-35% of rainbow trout stock developing ulceration in trout farming of Uttarakhand during summer seasons. The ulcer is confined to caudal peduncle region of dorsal aspect of the fish body, later on extending deep to the vertebrae. The infected fish were found dead within 3-4 weeks after the appearance of ulcer. The bacterial isolate from diseased rainbow trout has been identified as *A. hydrophila*, JX390650, Lab strain no. RTMCX1 on the basis of morphological, biochemical characteristics and phylogenetic analysis derived from 16S rRNA sequence. This RTMCX1 grows very fast and produces large, hemolytic, greenish colony on sheep & rainbow trout blood agar & is negative for indole production.

The provision of good challenge models is fundamental to study pathogenicity and treatment/control of aquatic animal diseases. The pathogenicity of *A. hydrophila*, JX390650, Lab strain RTMCX1 is confirmed in healthy rainbow trout by intra-peritoneal injection, and by survivability as well as growth of bacteria in trout serum. This strain has caused mortality in adult rainbow trout and showed high virulence compared to *Aeromonas veronii* (*ichthiosmia),* and *Aeromonas sobria*. The study has shown that *A. hydrophila* is a significant contributing factor to poor health and performance of rainbow trout under culture conditions, especially where temperature reaches upper end of the range of tolerance for this species, or when water quality is poor. The high susceptibility of rainbow trout to *A. hydrophila* may be related to the environment of this fish, which lives on clear running water, so less exposed and so less resistant to this pathogenic bacterium. The other reason may be that, this bacterium multiplies at very faster rate, so colonize and cause mortality very quickly in comparison to other pathogenic bacteria.

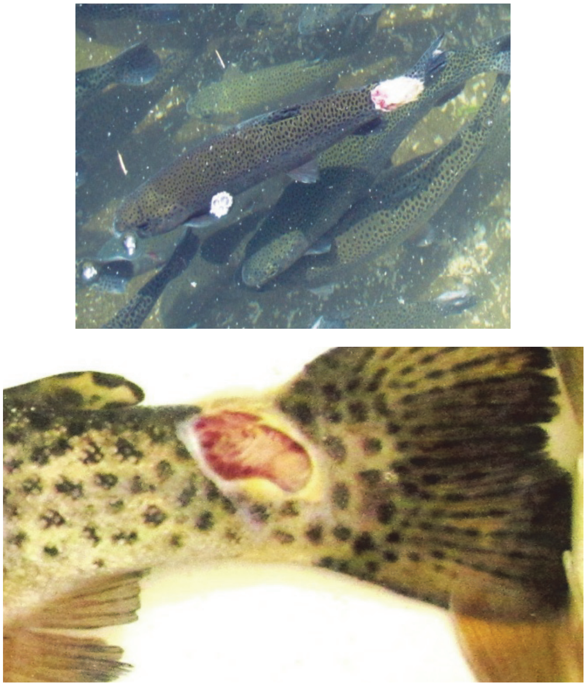

Figure1: Deep Ulceration in caudal peduncle region of Rainbow trout

(iv) Corneal Opacity in Coldwater Fish & Pathogenic Bacteria

Eye infection (at different stages) has been recorded in 20-25% of rainbow trout population weighing more 150 to 300g in farming conditions. The initial symptom is marked with hemorrhage in eye leading to corneal opacity and liquefaction within 2-3 months of infection. In advance stages of infections, conditions of unilateral & bilateral blindness are also raised in 65% of infected trout population. This eye infection has adverse effect on trout growth, reproduction & survival. Under present investigation, the weight loss due to eye infections in trout brooders is in a range of 30-40% in females & 40-50% in males. Four strains of *Lactococcus garvieae* (GenBank accession no: KM 604701, KM 604702, KM 604703 and KM 604704) are isolated and identified from diseased rainbow trout in trout farms of Uttarakhand and Himachal Pradesh suffering from corneal opacity. In a similar kind of infection, *Pseudomonas koreensis*, TPEB02, (NCBI JX390644) is recovered from golden mahseer eye, demonstrating corneal opacity. A preliminary experimental infection using golden mahseer fingerlings (11.5 $\pm$ 3.2 g, n = 21) indicated that a 12hr culture of *P. koreensis* is virulent to mahseer. The other bacterial flora isolated from eye infections of coldwater fish are *A. hydrophila, A veronii, Micrococcus luteus*, *Pseudomonas lurida & Acidovorax facilis*.

Figure 2: Hemorrhagic eye leading to corneal opacity & complete endothalmic condition in rainbow trout

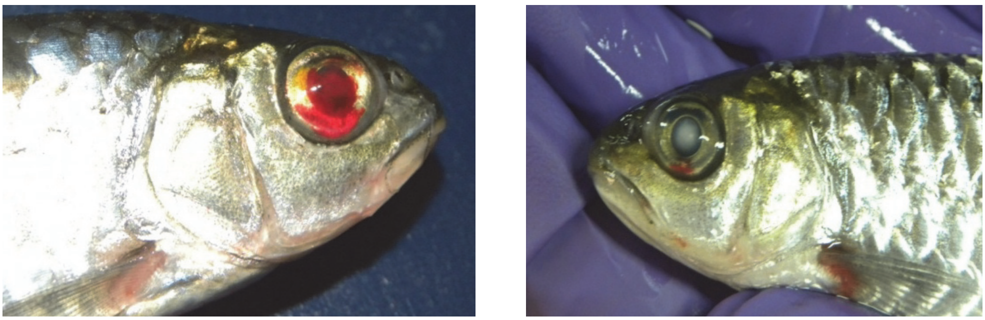

Figure 3: Hemorrhagic eye & corneal opacity in golden mahseer, *Tor putitora*

(v) Emerging, Facultative & Zoonotic Bacterial Pathogens in Coldwater: A Fact Needs Considerable Attentions

Citrobacter freundii has been frequently isolated from trout samples showing symptoms of tail rot, fin rot, gill rot & hemorrhage collected from different farms. Presence of *Corynebacterium* & *Caronobacterium* in trout & *detection of orange pigmented gram negative, rod shaped Chryseobacterium scophthalmum*, NCBI accession no. KM822770 *(13 isolates) in infected gills of golden mahseer collected from Bhimtal Lake* has raised the issues of emerging pathogens due to anthropogenic disturbances as well as climatic changes in coldwater environment. Many times we ignore bacteria pathogens of lesser known occurring in our culture environment. So this directorate has focused on concept of emerging bacterial pathogens in aquaculture to develop scientific information on host-pathogen interaction and virulence mechanism. Occurrences of facultative bacteria pathogens like *Escherichia coli, Micrococcus,* *Enterobacter, Shewanella, Morganela & Hafnia alvei* are also matter of concern in trout health management in farms of Indian Himalayan Regions. Data on occurrence of facultative pathogens in trout farms indicate that in adverse condition, they may cause possible disease outbreak leading to potential economic consequences for the trout farmers.

Apart from threat to fish, bacteria from coldwater environment may also pose a possible health threat to humans (zoonotic significance), as well as other terrestrial animals through the ingestion and handling of diseased fish and by exposure to aquatic environment. Presence of *Aeromonas hydrophila, A. veronii* (*ichthiosmia), Escherichia coli, Enterobacter, & Hafnia alvei* draws more attention for the effective control of bacteria of zoonotic importance through the administration of appropriate antibiotic, and also by improving the diagnostic measures through the use of advanced molecular technique. *Lactococcus garvieae* being zoonotic in nature, occurrence of this pathogen has possible health implication. It requires a strategic outlook executing proper prophylactic and therapeutic measures to control its infection in rainbow trout farms. Conducting awareness programme on zoonotic pathogens for trout handler/farmers is need of hour.

Figure 4: *Chryseobacterium scophthalmum (KM822770)* isolated from *Tor putitora* showing infected gill

(vi) Antibiotic Sensitivity of Coldwater Bacteria Pathogens

The antibiotic sensitivity of an isolate is required for effective clinical control, especially when the isolate is from diseased organism. Oxytetracycline and tetracycline are the most commonly used antibiotics in aquaculture to treat bacterial diseases, because they have broad spectrum of action and not very toxic to fish. The *Aeromonads* isolated from coldwater aquaculture are found resistant to penicillin and ampicillin. However, the sensitivity of the isolates of *Aeromonads* to majority of the antibiotics indicates the possibility of controlling bacteria by antibacterial agents in coldwater aquaculture. *Enterococci* isolated from eye infection in rainbow trout has shown resistant to Nalidixic acid(Na^{30}), Clindamycin(Cd^{2}), Lincomycin(L^{2}), Cephalexin(Cp^{30}), Polymyxin B(Pb[50]), Amphotericin B(AP[100]), Ceftazidine-Ca[30/10], Cloxacillin Cx[5], Amphotericin (AP[20]), Oxacillin (Ox[5]), Sulphamethoxypyridazine(St[300]), and Methicillin (Met[10]). Application of erythromycin has resulted isolates of *Entercocci* susceptible to the antibiotic. Recorded antibiotic susceptibility data for primary, secondary and other potential bacterial pathogen of rainbow trout and its culture environment may help us to develop a quick decision support platform indicating efficacy of antibiotic treatment (Dose & with drawl period), in case there is any bacterial infection in rainbow trout culture practices.

(vii) Parasitic Infections in Coldwater Aquaculture

(a) White spot disease in high altitude raceways reared Indian Snowtrout, *Schizothorax richardsonii*

The incident of white spot (Ich) infection is also observed in high altitude race-ways reared Indian Snowtrout, *Schizothorax richardsonii* in Uttarakhand. More than 60% of the stock of *S. richardsonii*, with an average length and weight of 10.5 cm and 31.6 gm respectively are found infected with white spot. Thorough morphological examination showed presence of white spot (lesions looking a small blister) on body surface including dorsal and caudal fins of fishes. Gills of infected fish are also observed with marked hyperplasia & presence of trophonts in the tissues. A successive (4 days) dip treatment trial of 50 ml L^1 formalin followed by 2% NaCl for 30 second is found effective in controlling the white spot recorded in dorsal body surface, fins and gills of the infected fish.

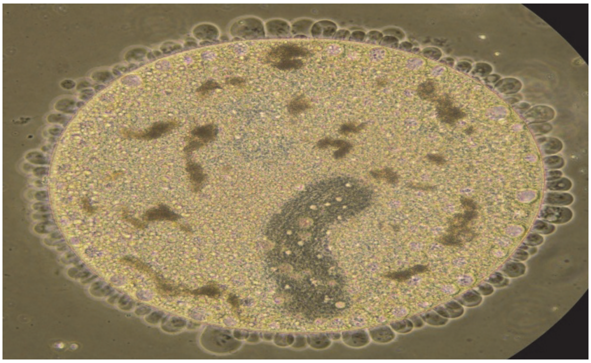

Figure 5: Trophonts, mature stage of *Ichthyophthrius multifiliis* isolated from Indian snow trout

(viii) Occurrence of fish louse (*Argulus* sp.) on Indian snow trout (*Schizothorax richardsonii*) and golden mahseer (*Tor putitora*)

Incidence of *Argulosis* is observed in Indian snowtrout, *Schizothorax richardsonii* and Golden mahseer, *Tor putitora* from cage culture unit of Bhimtal Lake, Uttarakhand. It is reported that 50% of *S. richardsonii* are infected with Argulus sp. with a mean intensity of infestation 2.06 & abundance 1.05. Similarly the stock infected in golden mahseer, *Tor putitora* is 25% with a mean intensity 0.34 and abundance 1.36. Maximum prevalence is observed during warmer months of the year with least in winter period. During the incidence of *Argulosis,* 1-2 % of fish stock is also observed with fungal infected. Measurement of crustacean parasite illustrated carapace length (CL) comprised 75% of average total body length (5.4 mm). The prevalence is positively correlated (r = 0.88) with decrease in water temperature. In recent years, it is observed that there is marked deterioration of water quality in Bhimtal Lake with recession of its margin and shallowing by anthropogenic activities. There is also a marked decrease in depth of the Lake during summer with increase in water temperature, appearance of aquatic weed infestation and muddy fauna. All these may be the basis for infestation of *Argulus* sp. on coldwater fish in cage culture system. The present infestation of *Argulus* sp. may be at initial stage, but it can be categorized in high-risk group, where organism is termed as typically pathogenic and requires immediate treatment and mechanical removal. Because this crustaceans infestation not only retards the growth of the fish, but also acts as a vector for entry of certain fish viruses and secondary infections such as bacterial and fungal diseases. Few researchers have also reported that due to *Argulus* infection, fish juveniles may get exposed to fungal and bacterial infections with skin irritation. This affects the feeding habit of the fish, resulting into retarding growth and sometimes death of the host. Thus a periodical monitoring program on fish health is considered necessary to keep the stock in healthy state. Virtually, there is no extensive survey on *Argulus* infection in coldwater aquatic resources of Indian Himalayan Region (IHR) conducted so far. So there is no information on distribution and abundance of *Argulus* infestation in coldwater bodies of India. The National Surveillance Programme on aquatic animal diseases may better address the issues of parasitic infections in coldwater aquaculture.

Figure 6: *Argulus* sp. infecting *Schizothorax richardsonii* & *Tor putitora in* cage rearing unit of Lake Bhimtal

(ix) Mycosis in Coldwater Aquaculture

The severity of mycosis in coldwater aquaculture is very much felt through different studies conducted in recent past. It is observed that an abrupt change in water temperature during winter months has resulted into fungal infection in *Tor putitora, Oncorhynchus mykiss*, *Cyprinus carpio*, *Schizothorax richardsonii* & in other coldwater fishes. A significant drop in water temperature from 22 °C to 4°C has shown to impair the fish immune system, causing loss and temporary suppression in mucus production in fish body. Mucous provides a physical barrier that prevents fungal spores infecting the skin of fish. Without mucus, skin is unprotected and fungal spores develop as masses of fungal hyphae that extend into the muscle tissue**.** In coldwater fishes, Saprolegniosis usually occurs between October and March when water temperature is below 22°C. *Saprolegnia diclina* and *Saprolegnia parasitica* infections remain more common in winter months. The fungal pathogens presently characterized are pathogenic oomycetes water moulds, *Saprolegnia diclina* (4 isolates) species responsible for the *Saprolegniasis* in Rainbow trout farms of Himachal Pradesh and *Saprolegnia australis* (3 isolates) from golden Mahseer in Uttarakhand.

Fungal infections have been difficult to prevent and treat. Formalin, hydrogen peroxide, Sodium chloride at different concentrations is recommended for the cure of fungal infected fish. A combination of good fish management and husbandry techniques involving proper handling of stock, ensuring hygienic environment with optimum feeding, stocking quality seed & maintaining regular water exchange may reduce chance of fungal infections in culture environment.

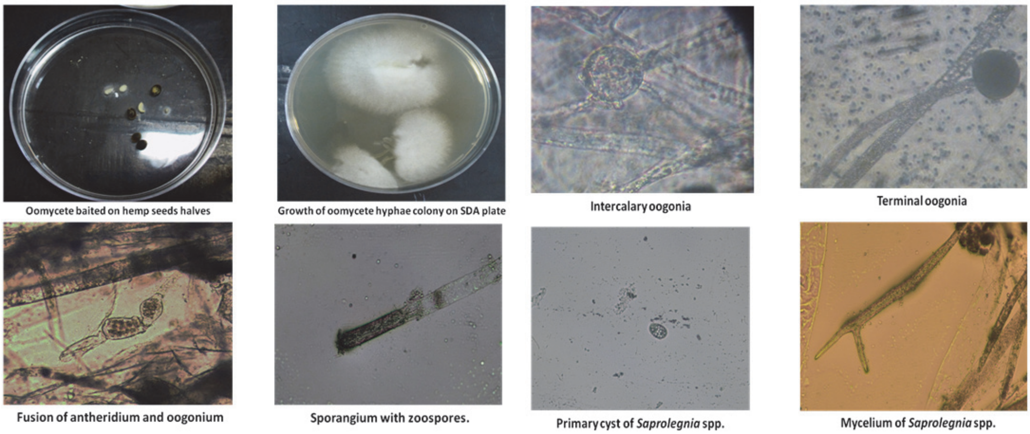

Figure 7:Developmental stages of Saprolegnia in coldwater farming

(x) Fish Health Management in coldwater: The recent advances

With establishment of modern diagnostic laboratories for viral, bacterial, parasitic & fungal pathogens, the directorate has initiated specific research programmes on fish health management and also participated in national surveillance programmes on aquatic animal diseases. The recent advances in fish health management in coldwater impart research activity on identification of viral signature in clinical samples or fish specimens involving fluorescent detection method, presently being carried out under national fellowship programme in the directorate. Development of nano-peptide based delivery system for effective drug delivery in coldwater fish is also under investigation. Seven antimicrobial synthetic peptides have been designed and synthesized from bacteriophage nucleotide and a large scale laboratory screening is under progress for their antibacterial characteristics against coldwater bacterial pathogens isolated from rainbow trout farms. Molecular approaches involving PCR diagnostic (16S rRNA, rpoD, gyrB gene) & approach of metagenomics have been under taken to detect pathogenic microbial flora in coldwater. Extensive survey has been conducted to list the chemicals, antibiotics & other aqua-drugs, presently used in coldwater aquaculture regions of our country. Efficacy & bio-safety issues of oxytetracycline application on rainbow trout & golden mahseer is under progress.

Conclusion

Coldwater fish being the inhabitant of high altitude wetland, lakes, rivers, torrential streams located in various geographical regions of our country possessing unique biological & physiological characteristics for their adaptation to cold climatic conditions, implementing an effective health management strategy has been realised difficult particularly in different artificially created culture practices. Beyond this, due to lack of mass scale culture practices, outbreak of any diseases is hardly evident. However, disease surveillance studies, has confirmed non availability of viral signature yet the presence of coldwater bacterial pathogens, *Flavobacterium psychrophilum, Renibacterium salmoninarum, Yersinia ruckerii & Aeromonas salmonicida* in our trout farms. Sporadic infections & mortality in coldwater fish farming have been attained with keen scientific effort, integrating traditional & advances in biotechnological approaches for effective fish health management.

Present Scenario of Aquatic Animal Health in Fisheries and Aquaculture of Andaman and Nicobar Islands

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Andaman and Nicobar Islands (ANI) is an archipelago situated in the South East of Bay of Bengal. ANI constitutes coastline of 1912 km, which is about one fourth of the coastline of India. The Exclusive Economic Zone (EEZ) around the Islands encompasses around 0.6 million km^2 which is around 30% of the total Indian EEZ (Arif, 1983 and Alagaraja, 1987). ANI represents one of the richest repositories of biodiversity in general and aquatic biodiversity in specific. The icthyofaunal diversity of ANI is constituted by 1434 species falling under 576 genera and 165 families of which, 400 species have commercial significance as food fishes (Rajan et al., 2013). Blessed with vast magnitude of fishery resources including coastal, pelagic, demersal, offshore, deep sea fishes and also high value marine organisms such as shrimps, lobsters, crabs, sea cucumbers and fishes such as tuna, groupers, snappers and ornamental fishes depicts its rich biodiversity (ANDFISH, 2005). Marine capture fisheries and aquaculture are the most prospective avenues for development in the Islands and marine fisheries is grossly underexploited.

Freshwater aquaculture plays a very important role in the livelihoods of local communities. Indian major carps, catfishes and freshwater prawn are mainly contributing to the total freshwater fish production in the Islands. At present, the Island consists of 2237 freshwater fish ponds with a total water spread area of 152.98 ha used for fish culture purpose and 7 reservoirs with 367 ha area. On an average, the Island produces about 193 tonnes/ year of freshwater fishes (Source: Directorate of Economics and Statistics, Andaman and Nicobar Administration, 2012-13). The Islands are unique compared to the rest of India in terms of the scope they offer for integrated development of culture fisheries in all the sectors viz. marine, brackishwater and freshwater.

The current trend in aquaculture development is towards increased intensification and commercialization of aquatic production with lack of proper health management measures. Many of the freshwater fishes are being imported from mainland to Island because of the consumption demand of settlers in the Islands. This is again of serious concern as the freshwater systems may be contaminated with pathogens by the fishes from mainland. A large number of diseases of fish and aquatic invertebrates have been reported worldwide. The aquatic environment of ANI when compared to mainland is also infested with some of the aquatic animal diseases. On the other hand, no systematic study on the diseases of wild and cultured fishes has been carried out to know the present health status of the aquatic animals of the Bay Islands. With this background, NSPAAD (National Surveillance Programme for Aquatic Animal Diseases) project has been initiated to detect and manage the aquatic animal diseases in ANI.

(I). Aquatic animal diseases reported from ANI

At present, only freshwater carp farming is being done in ANI while, brackishwater aquaculture and mariculture are the identified areas of development in the aquaculture sector. To some extent, the Island farmers import carp seeds from the mainland thus paving way for the entry of pathogens into this relatively virgin ecosystem. A vast number of serious diseases and pests of fish and aquatic invertebrates are known but majority of these are not described in the ANI and are thus exotic. As there are few research reports on the incidence of diseases, it is necessary to conduct a systematic investigation on the present state of health of the finfish and shellfish of ANI. The following table (Table 1) documents the aquatic animal diseases reported from ANI (Saravanan et al., 2015) before the implementation of NSPAAD project by ICAR-CIARI.

S. No.	Disease	Causative agent	Affected aquatic animals	References
1.	Acute form of abdominal dropsy	*Aeromonas hydrophila*	*Cirrhinus mrigala* (Mrigal)	Shome et al., 1996
2.	Atypical chronic form of abdominal dropsy	*A. hydrophila*	*Catla catla* (Catla)	Shome, 1999
3.	Pop-eye disease	*Vibrio species*	*Epinephelus spp*. (Grouper), *Caranx spp*. (Jack), *Triacanthus spp*. (Tripod fish), *Platax spp*. (Bat fish), *Balistapus undulatus* (Undulata trigger fish), *Pterois spp*. (Lion fish), *Chaetodon vagabundus* (Vagabond butterfly fish), *Canthigaster margaritata* (Pearl toby), *Naso spp*. (Tang fish) and *Lutjanus spp.*	Shome et al., 1999a
4.	Monogenetic trematode infection	*Diplectanum blairensis*, *Diplectanum jerbuae*,	*Sillago sihama* (Silver whiting), *Terapon jarbua* (Tiger perch) and *Lutjanus rangus* (Sweetlip	Gupta and Khanna, 1974

		Protancyrocephalus rangusi, Pseudoalitrematoides bengalensis and *Hammatopeduncularia arii*	snapper)	
5.	Digenetic trematode infection	*Gauhatiana lebedevi n. sp., Hypohepaticola andamanensis n. sp., Ectenurus antipodes, Lecithaster indicus, Ozakia tropica, Waretrema piscicola, Gyliauchen ozaki, Hexangium sigani, H. loossi, Bucephalopsis exilis, Prosorhynchus ozaki, Hysterolecithoides frontilatus, Hamacreadium interruptus, Mehracola ovocaudatum* and *Bivesicula australis*	Marine fishes mainly *Acanthurus sp.* (Surgeon fish), *Siganus vermiculatus* (Vermiculated spinefoot), *Rastrelliger kanagurta* (Indian mackerel), *Rastrelliger brachyosoma* (Shortbodied mackerel)	Gupta and Miglani, 1976; Hafeezullah and Dutta, 1980
6.	Trematode and cestode infection	*Elongoparorchis sp. Bothriocephalus sp.*	*Tachysurus sp.* (Marine catfish) and *Saurida tumbil* (Lizard fish)	CARI Annual Report, 1981
7.	White Spot Syndrome Disease	White Spot Syndrome Virus (WSSV)	Tiger shrimp (*Penaeus monodon),* Mud crab (*Scylla serrata)* and Banana shrimp (*Fenneropenaeus merguiensis)*	Sethi et al., 2011
8.	Laem-Singh Virus Disease	Laem-Singh Virus (LSNV)	Tiger shrimp (*Penaeus monodon)*	Kumar et al., 2011
9.	Luminescent Vibriosis	*Vibrio harveyi*	Tiger shrimp (*Penaeus monodon)*	Shome et al., 1999b
10.	Necrotic patches	Fungi mainly *Scolecobasidium sp.*	Corlas mainly *Porites* viz., *Porites lutea, P. lichen*. Others like *Montipora tuberculosa, Goniopora sp.* and *Goniastra sp.*	Ravindran et al., 1999; Raghukumar & Raghukumar, 1991
11.	Bleaching of corals	Elevated Sea Surface Temperature (SST)	*Acropora formosa, Acropora nobilis, Acropora robusta, Acropora breuggemanni* and	Krishnan et al., 2011

	Acropora grandis

Table 1. Aquatic animal diseases reported from ANI before the implementation of NSPAAD project

(II). Significant achievements under NSPAAD project

ANI are believed to be free from many fish and shellfish diseases when compared to the mainland India and other neighbouring countries though it shares close proximity with South East Asian countries like Indonesia, Thailand and Malaysia where many types of diseases were reported. The absence of many diseases may be due to two reasons, either due to geographical isolation of the Islands or may be due to lack of intensified research on disease surveillance of aquatic animals. With this background, NSPAAD project has been initiated since 2015, to carry out aquatic animal disease surveillance in ANI.

Being one of the remotest sub-centres under NSPAAD, surveillance of freshwater and marine finfish and shellfish diseases is being conducted covering all the three districts of Andaman and Nicobar Islands (ANI). Baseline data has been collected from a total of 161 freshwater fish farms and necessary guidance were provided to the farmers. Two parasitic outbreaks of *Argulus sp.* and *Myxobolus sp.* were reported for the first time from the freshwater fish farms of ANI. Occurrence of branchial parasitic cymothoid, *Ryukyua circularis* from the marine finfish, *Amblygaster sirm* and another parasitic cymothoid, *Norileca indica* from carangid fish, *Selar crumenophthalmus* were reported for the first time from ANI. Likewise, marine super giant isopod, *Bathynomus lowryi* (Isopoda: Cirolanidae) was also reported for the first time.

A total of 647 freshwater fish and prawn samples, 353 shrimp samples, 291 grouper samples and 20 gold fish samples were collected for disease screening. All the collected freshwater fish and prawn samples were negative for KHV, SVC, MrNV and XSV. Grouper samples were negative for VNN and RSIVD. All the gold fish samples gave negative result for CyHV-2. Shrimp samples (*Penaeus monodon*) collected from Lohabarrack, Betapur and Campbell Bay landing centres gave positive result for IHHNV. Likewise, shrimp samples *(P. monodon)* collected from Lohabarrack, Junglighat and Campbell Bay landing centres gave positive result for WSSV. All the tested shrimp samples gave negative result for MBV, EHP, AHPND, IMNV, YHV and TSV.

(III). Important aquatic animal diseases reported from ANI and adjoining nations

Diseases are the major constraints for the development of aquaculture sector. Compared to the nearby countries and mainland India, very few aquatic animal diseases have been reported from ANI. Abdominal dropsy from freshwater fishes, pop-eye disease, trematode and cestode infestations from marine fishes, WSSV, IHHNV and luminescent vibriosis from marine crustaceans have been reported from the Bay Islands. The magnitude of diseases reported from the Islands is very less when compared to the deadly diseases present in other countries (Table 2).

Aquatic Animal Diseases	**Indonesia**	**Thailand**	**Malaysia**	**Sri Lanka**	**Myanmar**	**India**	**Andaman & Nicobar Islands**
	Freshwater						
Viral Diseases							
Koi Herpesvirus (KHV) disease	+	+	+	#	✘	✘	✘
Spring Viremia of Carp (SVC)	✘	✘	✘	✘	✘	✘	✘
White tail disease	+	+	+	#	#	+	✘
Bacterial Diseases							
Haemorrhagic Septicaemia	+	#	+	+	+	+	✘
Abdominal dropsy	+	+	#	+	+	+	+
Edwardsiellosis	+	+	+	#	#	+	✘
Parasitic Diseases							
Ich or white spot disease	+	+	+	+	#	+	✘
Trichodiniasis	+	+	+	+	#	+	✘
Lernaesis	+	+	+	+	+	+	✘
Argulosis	+	+	+	+	+	+	+
Gill and skin fluke infection	+	+	+	+	+	+	✘
Fungal Diseases							
EUS	+	+	+	+	+	+	✘
Saprolegniasis	+	+	#	+	+	+	✘
	Marine						
Viral Diseases							
Viral nervous necrosis (VNN)	+	+	+	#	#	+	✘
Grouper Iridoviral disease	+	+	+	#	#	✘	✘
White Spot Disease	+	+	+	+	+	+	+
IHHN	+	+	+	+	#	+	+
Taura Syndrome	+	+	+	#	✘	✘	✘
YHD	+	+	+	+	✘	+	✘
MBVD	+	+	+	+	#	+	✘
Bacterial Diseases							
Vibriosis	+	+	+	+	+	+	+
Necrotizing Hepatopancreatitis	✘	✘	✘	✘	#	✘	✘

Streptococcosis	+	+	+	#	#	#	×
Parasitic Diseases							
Trematode and Cestode infestation	+	+	+	+	+	+	+
Marine white spot (Cryptocaryon irritans)	+	+	+	#	#	#	×

+ Disease reported × Disease not reported # Information not available

Table 2. Important aquatic animal diseases reported from ANI and adjoining nations

(IV). Biological invasion threats in ANI

The main threat to the Island aquaculture in biosecurity point of view is the import of carp seed from mainland which leads to the invasion of pathogen from mainland to the virgin Island ecosystem. The cyprinids like *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*, *Cyprinus carpio* and *Ctenopharyngodon idella* were farmed based on local consumption and demand by the local settlers, which had contributed in introduction of other small weed fishes, which is said to be introduced along with carp spawns from mainland (Rema Devi, 2010). Invasive species can give a tough competition for food and niche with native species. This alien species at all life stages competes with native fishes for food. Native fish that rely upon insects, fish, crustaceans, frogs could face reductions resulting from the loss of food and also it is obvious for the native fish being predated by this invasive fishes. Due to the unregulated aquaculture practices and import of illegal fish seeds from Kolkata by farmers, the fate of the Island ecosystem is really under threat. This situation of biological invasion will definitely have an impact on the food chain of the native species. Thus, introductions of aquatic animals to the region are always accompanied by the risk of the introduction and establishment of serious exotic diseases.

Invasive species also acts as a media for the transfer of pathogens and also possess a great threat to the biodiversity of ANI. With the ongoing aquaculture activities in the Islands, there is more chance of spread of disease from the non-native stock to the native aquatic biodiversity and also there is further room for spread of pathogens to the existing aquaculture activity. As the aquaculture industry continues to grow, the threat of infectious diseases to fish production facilities will continue. Since it is very difficult to hinder the devastating effect of anthropogenic activity and moreover number of alien species continue being transported in these small Islands for aquaculture practices, these organisms could replace regionally unique organisms resulting in the loss of overall diversity that defines bio-geographic regions (Scott & Helfman, 2001).

Increasing trend of new pathogens is being detected worldwide and emerging as threats to new locations. While the risk of disease in production settings cannot be completely eliminated, the use of biosecurity measures on the farm can help to prevent disease introductions and spread. In such backdrop, the fragile ecosystem of ANI is under constant threat and is in need of strict quarantine and biosecurity measures.

(V). Conclusion

Freshwater aquaculture can be a well-paid business in ANI due to its high demand and also offers sufficient employment generation to rural youth and thus can serve as a source of livelihood. The pristine aquatic environment of ANI when compared to mainland is also infested with some of the aquatic pathogens. The screening of fishes entering from mainland is an important aspect to be considered in biosecurity point of view. On the other hand, the systematic study on the disease status of wild and cultured fishes has been carried out to know the present health status of the aquatic animals of the Bay Islands. Few deadly aquatic diseases like IHNNV, WSSV and parasitic infestations were reported from ANI after the implementation of NSPAAD, which supports the fact that comparatively less number of diseases reported earlier might be due to lack of intensified research on aquatic animal disease surveillance in ANI. Detailed information on the aquatic animal diseases would lead to the development of policies and plans to check the introduction and spread of diseases into this relatively virgin ecosystem. Ultimately, disease free aquaculture practices and healthy aquatic ecosystem leads to the improved production for the famers and also sustain its resources for the future generation.

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Strategic Role of ICAR-National Bureau of Fish Genetic Resources, Lucknow in Fish Genetic Resource Management with Focus on Preventing Aquatic Animal Diseases

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The fish genetic resource management was institutionalized with the establishment of National bureau of Fish Genetic Resources during 1986 under the aegis of Indian Council of Agricultural research, Department of Agricultural Research and Education, Ministry of Agriculture, Government of India. ICAR-NBFGR is a specialized organization mandated to conduct research related to fish genetic resource conservation and management. India is among few who took the lead to accelerate scientific research, evaluation, assessment and conservation of valuable fish diversity by establishing a National Bureau of Fish Genetic Resources (NBFGR) in1983. The institute has since then made valuable contributions and has become a focal point for technical support on fish genetic resources in the country. Besides exploring, collecting, conserving and utilizing FGR for increasing aquaculture productivity, genetic resources management today encompasses a multilevel domain of scientific, policy, social, ecological, legal and financial aspects. Various stakeholders are included in this process of FGR management viz., fisherfolks, fish farmers, communities, breeders, researchers, managers and policy makers. Moreover, FGR management involves interaction at global level with the countries and inter-governmental platforms like the Food and Agriculture Organization of the United Nations (FAO), Network of Aquaculture Centres in Asia pacific (NACA), Secretariat of the 'Convention on Biological Diversity' (CBD, 1993), 'Commission on Genetic Resources for Food and Agriculture' (CGRFA), FAO 'Code of Conduct for Responsible Fisheries' (CCRF, 1995), 'FAO Committee on Fisheries' (COFI) and so on. Several international treaties such as the CBD, World Trade Organization (WTO) Agreement, and national laws such as Biological Diversity Act, 2002, are the most important ones.

Global thrust on genetic resource management stems from the realization that natural biological diversity is a critical basic ingredient to enhance food production for growing population. The action plans implemented by Convention of Biological Diversity (CBD), as Aichis biodiversity targets are equally relevant to all components of agrobiodiversity including aquatic genetic resources. The genetic diversity under water, be it freshwater, brackishwater or marine ecosystem, have unique significance towards bridging the food availability gap, nutritional, international trade and livelihood security. Agrobiodiversity with respect to fisheries is equally dependent upon capture from wild resources and aquaculture production. However, with marine capture fishery has reached plateau while aquaculture rising @ 9% consistently for the last two decades, becomes a reliable hope for enhancing fish production. This emphasizes the need of sustainable utilization and management of the FGR's for their use in aquaculture improvement and providing resilience against climate change.

ICAR-NBFGR has been consistently working on developing in-house capacity and tools to address researchable issues to generate knowledge on FGR of India, their genetic diversity, conservation and sustainable utilization and effective decision making on introduction of exotic fish species for culture and prevention of exotic fish diseases. The bureau has capacity to conduct research in the areas of explorations and cataloguing of FGR, characterization, development of genomic resources, conservation genetics and genomics, *ex-situ* conservation through sperm and cell cryopreservation, evaluation of germplasm, risk assessment due to exotic species and pathogens. This make the organization responsible for providing technical backstopping to the policy making authorities with respect to decision making on various issues of conservation and management, germplasm exchange and introduction of exotic species in the country.

India has rich Fish genetic resources and has high level of endemism too. Therefore the resources available in aquatic system are important not only for utilization in food production system but also for various research activities and many countries will be interested to access these resources. However, post CBD and Nagoya protocol, the countries have sovereign rights on these resources and regional cooperation will have a special role to play in sustaining benefits from the access to these resources. Documentation of this knowledge is an important aspect. In this global scenario, institutionalization of FGR management, achieved in the form of ICAR-NBFGR, way back in 1986 by Government of India, should be seen as visionary step.

India possesses near 10% of the global biodiversity in terms of fish and shellfish species. The discoveries of 8 fish species which are new to science, during last 2 years, by ICAR indicate that the fish agrobiodiversity can be still expanded through detailed explorations in the country. The diversity gives new opportunities of diversification of aquaculture, which can be resilient to changing climates and useful for livelihood of stakeholders. It becomes pertinent that some of these new species to be evaluated for their potential for enhancing aquaculture production.

One of the important aspects of FGR is lack of adequate knowledge on intraspecific genetic diversity. In contrast to plant and animals, where breeds and varieties are defined and are put to use. ICAR-NBFGR has been successful in working in this direction and has characterized 23 fish species, of aquaculture and conservation value, for their genetic stocks. Now for future work program, important genetic stocks of aquaculture stocks need to be evaluated for their on-farm performance, so that the useful traits can be utilized and enhancing farmer's income. Commission on Genetic Resources for Food and Agriculture (CGRFA) under FAO has recognized the lack of such knowledge as major impediment in the blue growth of aquaculture, worldwide. Genetic stock knowledge is also a critical input for strategic planning for *in situ* conservation and stick enhancement of threatened wild relatives of aquaculture species and harvested species in their native distribution range. ICAR-NBFGR in its future program will establish standardized and validated molecular marker panels, for such important species for enhancing the pace of knowledge generation on fish genetic stocks. It is also quite encouraging that FAO Commission on Genetic Resources for Food and Agriculture (CGRFA) is developing country-wise status reports on aquatic genetic resources. The scientific knowledge developed by ICAR-NBFGR was a pivotal component in the status report submitted from India. This is part of establishing a global action plan on aquatic genetic resources. In view of several national and international regulatory developments, this will help to focus on the sustainable management of genetic resources by initiating systematic science-based efforts, from their exploration, use and conservation.

With the advent of genomic technologies, a vast canvas becomes open to find trait related genomic architecture and mechanism controlling these physiological mechanisms. The markers associated with such traits can be useful link to establish fast genetic improvement programs. ICAR-NBFGR has timely established in-house capacity for such work and whole genome sequencing of 3 finfish species, rohu, catfish and hilsa and a fungal pathogen is in advanced stage of completion. More such species will be implemented so that genomic level information of Indian FGR is available.

Ex situ conservation and repositories are an important aspect of FGR management. These are useful for conservation, utilization of material for future research, and claiming IPR stake. ICAR-NBFGR has been working on these aspects. In India, NBFGR is the primary organization carrying out fish sperm cryopreservation for long-term gene banking. The fish sperm cryopreservation needs development of species-specific protocols. Species-specific sperm cryopreservation protocols have been developed for over 28 species. Fish gamete cryopreservation research still faces an important challenge in the form of long-term storage of fish eggs and embryos except the minute fertilized abalone eggs. To overcome this issue, thrust is being given on stem cell preservation. Success is achieved on model species through surrogate broodstock development and now future program will be established on aquaculture species to help diversification. These cells will be important to help preserving diploid germplasm, not possible with sperm. NBFGR has established a National Fish Cell Line

Repository which provides cell lines to researchers in the country on demand. There will be future effort to establish an integrated fish germplasm repository which can serve as reference point for FGR including voucher specimens for taxonomy, and other registered accessions. Live germplasm resource centres for prioritized species will also be an important area of work for conservation aquaculture.

In India, the fish and fish products are at present, the largest group in agricultural exports, with close to 10.00 lakh tonnes in terms of quantity and Rs.30,000 crores (4700 million USD) in value with shrimp being a major commodity. This accounts for around 10% of the total exports of the country and nearly 20% of the agricultural exports. Over 80% of the production is supported by an exotic, white leg shrimp and the seed production dependent upon the import of broodstock and multiplication. ICAR-NBFGR has consistently supported this sector, through its research on disease diagnostics, surveillance program and technical backstopping for effective implementation of prevention of disease outbreaks and accurate reporting of diseases. ICAR-NBFGR supported DADF with guidelines to establish quarantine facility to comply with WTO regime. Research on disease diagnostic protocol need to be strengthened so that more species under culture systems can be assessed. Risk and benefit assessment model that suites need of India need to be established and validated for future use in assessment of exotic introductions.

Early detection is considered to be key to the control of diseases. This requires active vigilance for signs of disease outbreak, rapid diagnosis of its infectious aetiology and can only be achieved through a structured surveillance programme. Considering the necessity of a surveillance programme in the country, a National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) was initiated in 2013, which is being funded by the National Fisheries Development Board (NFDB), Hyderabad; Department of Animal Husbandry, Dairying and Fisheries (DADF), Ministry of Agriculture, Government of India. At present, the programme is being implemented in 16 states of aquaculture and fisheries importance and two Union Territories through 27 National/State Fisheries Research Institutes/colleges, with ICAR-National Bureau of Fish Genetic Resources (NBFGR), Lucknow as the Co-ordinating institute.

All the information generated under NSPAAD in predesigned formats (baseline information of the farms, biological sample collection information under active surveillance which includes both normal and abnormal observations, disease outbreak information of finfish, crustaceans and molluscs) are being compiled in a National Aquatic Animal Disease Database. Many new pathogens of aquatic animals have been reported for the first time from country.

- Infection with cyprinid herpesvirus-2 in goldfish
- Infection with carp edema virus in koi carps

- Infection with *Enterocytozoon hepatopenaei* (EHP) in cultured shrimps
- Infectious myonecrosis in cultured shrimps
- Infection with *Perkinsus olseni* in a new host, *Perna viridis*

A vital component of the surveillance programme is competent and reliable diagnostic laboratory support that is fully integrated into the overall disease surveillance programme. ICAR-NBFGR has the diagnostic capability for all the OIE listed as well as emerging diseases of aquatic animals. The positive controls are being provided to the collaborating centres for strengthening the diagnostic facilitates in the the country. A "Diagnostic Manual for Aquatic Animal Diseases of National Concern" containing information about etiological agent, susceptible host species, target organs, diagnostic methods etc. for all the prioritized diseases has been published.

The importance of antibodies in health management measures such as diagnosis, identification, stereotyping, antigen characterization, epidemiology and vaccine development is well known. Traditionally, polyclonal antibodies raised in rabbits are used in disease diagnosis, stereotyping and other biological applications. However, due to the limitation of polyclonal antisera such as cross reaction, limited quantity, unwanted background reactions and inability to discriminate antigen at epitope level, Monoclonal antibodies (MAb) are becoming increasingly popular. The usefulness of monoclonal antibodies stems from three characteristics- their specificity of binding, homogeneity and ability to be produced in unlimited quantities. The monoclonal antibodies to fish immunoglobulins can be used as secondary antibody in immunoassays and hence have immense value in knowing the health status of candidate species.

Aphanomyces invadans, causative agent of Epizootic Ulcerative syndrome has been isolated and maintained. Resurgence of EUS has been documented from several parts of the country.For understanding host-pathogen interaction in dynamic disease situation, susceptible rohu and resistant common carps infected with *Aphanomyces invadans* are being studied for dual RNAseq to understand mechanisms underlying susceptibility/resistance. Deciphering *Aphanomyces invadans* genome to understand its mechanism of infection in fishes is underway. Genome sequencing resulted in the generation of 20.6 Gb polymerase read data and 12.45 Gb read of insert data. The assembly of the data resulted in primary assembly of 62Mb with 396 contigs. Genome completeness analysis indicated that 98% of the core eukaryotic genes are correctly predicted. The de novo gene prediction showed a total of 18622 genes and for pathway analysis, 18622 protein sequences have been annotated. Completion of RNASequencing of three different stages *viz*. zoospore, germling and mycelium of *A. invadans*. Two antigenic preparations ((immunization with inactivated germlings of *A. invadans*, inactivated infective germlings + adjuvant (montanide)) were evaluated for their protective efficacy against *A. invadans* infection. The group injected with inactivated germlings of *A. invadans* + adjuvant (montanide) showed 66% relative percent survival.

Selective Breeding as an Effective Tool for Disease Management

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Aquatic animal health management is a critical pre-requisite for any sustainable aquaculture program. Aquatic system, if not managed properly offers opportunities for pathogens to thrive leading to the prevalence of diseases. Immense economic losses arise from fish epidemics, and tackling such epidemics is a major task faced by aquaculturists. India produces 7 million tonnes of fish and shellfish and earns Rs. 1,00,000 crore annually through exports of fisheries products. However, the annual loss due to diseases in aquaculture was estimated to be approximately Rs. 600 crore (source: The Hindu, 11 August 2011) which proves the direct impact of diseases contributing unprecedented economic loss to the country.

The losses due to infection by protozoan parasites in carp nurseries and owing to the menacing *Argulus* in grow-out ponds are well known but no records are available in monetary terms. The impact of Epizootic Ulcerative Syndrome (EUS) that wiped out a number of species in rivers and reservoirs, tanks and ponds and a wide variety of water bodies is green in our memories. Yet, despite the hue and cry all over the country, no efforts were made to monitor and contain the losses suffered year after year in economic terms except in some pockets

Diseases are a primary constraint to sustainable aquaculture production and product trade; it has a direct impact on biodiversity, socio-economic status and international trade. Moreover, with the increasing demand of high quality and safe aquaculture products and its direct role in providing economic security, aquatic animal health management has impressed upon the aquaculture scientists to adopt newer technologies and enhanced enforcement of regulations for better governance of aquaculture.

Implementation of technologies

India has been pursuing blue revolution for several decades through inter-collaboration of technologies. The necessity of more sensitive pathogen detection tools to prevent diseases before they spread and identification of disease-resistant strains should be our priority to ensure disease free seed, marketable fish, brood stock and better production. In the current scenario, it has become critical to analyse the sources, causes and mechanisms of fish disease processes through accurate diagnosis for adequate disease management. The spread of any primary pathogen in cultured fish population needs identification at early stages for effective governance and risk analyses. Therefore, the diagnostic and pathogen detection technologies are essentially required to combat losses. While chemicals were and are used to control diseases with whatever be the degree of effectiveness, vaccines – which are not familiar in India so far – are far more effective control measures. Vaccines are used on a large scale to control Salmon lice and other diseases, in both Norway and Chile. However, modern tools involving genetic improvement through selective breeding are also being used to develop resistance to diseases – whether parasitic, fungal, bacterial or viral. Immunoassays and nucleic acid probes are now available for specific pathogen screening, in addition to the conventional techniques of disease diagnosis. However, these techniques are yet to find its way from lab to the field as routine diagnostic kits.

Genetics of disease resistance

The success and sustainability of aquaculture industry largely depend on disease prevention and control and genetic improvement of fishes for disease resistance can be a feasible alternative to prevent disease outbreaks. Genetic variation of immunological and physiological parameters could also be the determining factors when correlated with disease resistance. To augment the economic returns and to control the prevalence and emergence of diseases, genetic improvement programs are being taken up worldwide where all commercially valuable traits have been incorporated. In India, the selective breeding program involving rohu, *Labeo rohita*, on improving the growth rate as the major objective has resulted in 'Jayantirohu' that has shown growth improvement (17% per generation). 'Jayanti' is now being further developed for resistance against *Aeromonas hydrophila*– a high-density SNP (linkage map) was generated and QTLs (Quantitative Trait Loci) identified – the product is now being tested in field conditions.

Selective breeding for disease resistance with the knowledge of inheritance of the traits gives accurate and reliable results. With selective breeding other tools such as molecular markers and association studies are very helpful in generating accurate information where the characters such as resistance against diseases is governed by multiple genetic and genetic factors. The new technologies such as next generation sequencing and high throughput genotyping are helpful in identifying variants for characters that might help in identifying the potential disease free brooders.

A New Approach

Disease outbreaks are recognised as a serious threat to aquaculture production and trade worldwide because of its environmental-ecological and socio-economic effect on livelihood. The most important part of health management is to understand origin of disease and its transmission. The barriers that may be physical, chemical or biological create a safeguard for the cultured fishes but these barriers should not have harmful effects on it. Now is the time to put concerted efforts to attain near-to-perfect disease-free fish population through science and innovation. There is an urgent need to facilitate the exchange of knowledge, innovations, difficulties and ideas between scientists, entrepreneurs, exporters, state fisheries officials and farmers which will ultimately contribute to productivity enhancement of the system. The aquaculture industry lacks a network of effective disease control system in the country. Therefore, it is imperative to have a central regulatory body that can guide and regulate the implementation of technologies, certification procedure, quarantine measures and quality control of the stock.

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Executive Summary

National Surveillance Programme for Aquatic Animal Diseases

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Surveillance programme for monitoring and controlling spread of diseases of national and international concern has become a primary requirement for effective health management and ultimately for sustainable aquaculture. Considering the importance, a National Surveillance Programme for Aquatic Animal Diseases is being implemented in 16 states and 2 Union Territories through 27 collaborating centres. The programme is funded by National Fisheries Development Board, Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture and Farmers Welfare, Government of India and is being coordinated by ICAR-National Bureau of Fish Genetic Resources, Lucknow. The major emphasis of the programme has been to strengthen the passive surveillance system in the country, so that there is improvement in the disease reporting by the farmers and state fisheries officers. In this direction, a total of 340 awareness programmes have been organized in which 12342 stakeholders including fish farmers and state fisheries officers participated. In addition, to strengthen the diagnostic capability of state fisheries officers, a total of 31 trainings have been organised in which 611 stakeholders were imparted training on fish disease diagnosis. In addition, for sensitizing all the principal investigators and co-investigators of all the collaborating centres, an orientation training workshop, involving international resource persons and a FAO Re-echo Seminar on acute hepatopancreatic necrosis disease (AHPND) have been organised. In addition, active surveillance is being undertaken in about 110 districts and over 1100 farms are being monitored. Each selected farm is visited twice per crop for collection of samples and screened for selected pathogens viz. spring viremia of carp virus (SVCV) & koi herpesvirus (KHV) for carps; infectious pancreatic necrosis virus (IPNV) & viral haemorrhagic septicemia virus (VHSV) for coldwater fishes; yellow head virus (YHV), infectious myonecrosis virus (IMNV), taura syndrome virus (TSV), infectious hypodermal and haematopoietic necrosis virus (IHHNV) & white spot syndrome virus (WSSV) in shrimps and *Perkinsus olseni*, *Marteilia refringens*, *Bonamia ostreae*, *B. exitiosa* & *Oyster herpesvirus* for molluscs.

Importantly, SVCV, KHV, IPNV, VHSV, YHV and TSV have not been detected till date. A national database on aquatic animal diseases is also being developed. Under the programme, for investigating new disease outbreak, there is a provision for constituting emergency response team comprising scientists from different institutes to carry out disease investigation. Following confirmation of a new disease, the same is validated in at least 2 more collaborating centres and a detailed report is submitted to the Competent Authority i.e. DADF. Till date, such teams have been constituted for dispelling speculations and misconceptions regarding occurrence of the AHPND in the country, investigating large-scale mortalities in goldfish in Hooghly District, West Bengal as well as for knowing the spread of IMN. For AHPND, none of the screened samples were found to be positive, whereas, infection with Cyprinid herpesvirus-2 was found to be responsible for the mortalities in golfish. Studies are under progress to know the spread of IMN. During the reporting period, four new pathogens viz. cyprinid herpesvirus-2, carp edema virus, *Enterocytozoon hepatopenaei* and infectious myonecrosis virus have been detected. Moreover, *Perkinsus olseni* has been detected in a new host i.e. green mussel, *Perna viridis* for the first time. In order to follow uniform protocols for diagnosis, a Diagnostic Manual for Aquatic Animal Diseases of National Concern for all the prioritized diseases has been published. Furthermore, diagnostic capability has been developed for OIE-listed and emerging pathogens of finfish and shellfish. The programme has improved in reporting of aquatic animal diseases to international organisations viz. NACA and OIE. The implementation of NSPAAD is helping to know range and distribution of pathogens affecting aquatic animals in the country which would be useful for better targeting of efforts to control and reduce the risk of spread of aquatic animal diseases.

Judicious Use of Drugs and Antimicrobials in Aquaculture

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The importance of aquaculture in meeting the growing demand for fish cannot be overemphasized. According to FAO estimates, during 2014, global fish production was 167.2 million tons, of which 146.3 million tons were food fish. Global aquaculture production was 73.8 million tons with an estimated first sale value of $160.2 billion. Thus aquaculture contributes to about half of global food fish production. Interestingly, nearly 89% of global aquaculture production comes from Asia with China dominating the production contributing to nearly 62% of global production. Other major aquaculture producers in Asia include India, Vietnam, Thailand, Bangladesh, Indonesia, Myanmar and Philippines. Fish is also one of the highly traded commodities and in 2014, fish exports touched $148 billion. While developing countries in Asia were major producers of aquaculture products, developed countries constitute the major market. The largest market share is held by the European Union ($54 billion) and the combined market share with United States of America and Japan is 63%.

Rapid growth of aquaculture during the last two decades has not been without challenges. Mortality due to diseases has been one of the greatest challenges and this has been accompanied most often by the over use of chemicals and drugs. Most recent has been the outbreaks of bacterial disease, Acute hepatopancreatic necrosis disease (AHPND) in shrimp. The selection and spread of antibiotic resistance due to indiscriminate use of antibiotics in various sectors including aquaculture has been drawing attention of agencies involved in public health as food safety regulators and consumers. FAO/OIE/WHO Expert consultation on antimicrobial use in aquaculture and antimicrobial resistance identified the following hazards associated with antimicrobial use in aquaculture (a) antimicrobial residues and (b) antimicrobial resistance (FAO/OIE/WHO, 2006).

It is now agreed that antibiotic resistance determinants are ancient in origin and can be found in bacteria from pre-antibiotic era. Genes encoding resistance to β-lactam, tetracycline and glycopeptide antibiotics could be found in bacteria from 30,000 year old Beringian permafrost sediments. At least some of the resistance determinants that are presently circulating among human pathogens have been thought to have originated from environmental bacteria. For example, *qnr* genes encoding quinolone resistance and found in plasmids of *E. coli* and *Salmonella* might have originated from aquatic organisms like *Shewanella* or members of aquatic Vibrionaceae, where the *qnr* gene is found in the chromosome. But indiscrimate use of antibiotics in agriculture, animal husbandry and medical sectors has led to massive increase in the proportion of resistant bacteria in different sectors. It is well accepted that antibiotic resistance determinants do not respect phylogenetic or geographical borders. Therefore any action to reduce risk due to resistant bacteria should involve integrated action involving environment, agriculture, environment and medical sectors.

The Global Action Plan on antimicrobial resistance (A68/20) of the World Health Organisation has been adopted at the 68th Session of the World Health Assembly as resolution WHA 68.7 in 2015. This Global Action Plan has five strategic objectives: (1) Improve awareness and understanding of antimicrobial resistance through effective communication, education and training (2) Strengthen the knowledge and education basis through surveillance and research (3) Reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures (4) optimize the use of antimicrobial medicines in human and animal health (5) Develop the economic case for sustainable investment that takes account of the needs of all countries, and increase investment in new diagnostic tools, vaccines and other investment.

FAO member countries, during 39th FAO Conference adopted resolution 4/2015 in which FAO action plan on antimicrobial resistance has been outlined. FAO action plan has four focus areas (a) improve awareness on antimicrobial resistance and related threats (b) develop capacity for surveillance and monitoring of antimicrobial resistance and antimicrobial use in food and agriculture (c) strengthen governance related to antimicrobial resistance and antimicrobial use in food and agriculture (d) promote good practices in food and agriculture systems and prudent use of antimicrobials. FAO member countries are expected to implement programmes in line with these focal areas.

Experts' Profile

Prof. K. Larry Hammell is a Professor and Associate Dean, Graduate Studies and Research in University of Prince Edward Island, Canada. His areas of expertise include aquaculture health and production management, disease surveillance in aquaculture, clinical field trials in aquaculture, health and production risk factor studies for fish farms. His institute is one of the OIE collaborating centres for diseases of aquatic animals particularly on Epidemiology and Risk Assessment of Aquatic Animal Diseases.

Prof. Kenton Lloyd Morgan is currently working as Emeritus Professor, Epidemiology, University of Liverpool, United Kingdom. He was the Professor since 1996 till 2016. He is a Visiting Professor, University Southern Chile; University of Zimbabwe, Harare; Member, sea lice research advisory committee, Member, communicable diseases task force, Liverpool. He is a Fellow of Royal College Medicine, Member of Royal College Veterinary Surgeons and Chairman/Member of several societies viz. British Society Animal Science, British Camelid Veterinary Group etc. He is a foundation diplomat of the European College of Veterinary Public Health and an honorary member of the Society of Veterinary Epidemiology and Preventive Medicine. He is a Member in the editorial board Preventive Veterinary Medicine; Editor, Epidemiology and Infection. He has supervised over 30 PhD students and published more than 180 papers.

Dr. Flavio Corsin is Ph.D. in aquatic epidemiology from the University of Liverpool with research in rural areas in Vietnam and India. Afterwards, Dr. Flavio worked for the Network of Aquaculture Centres in Asia-Pacific (NACA) during which he coordinated several projects in Vietnam, Iran, Indonesia and other Asia-Pacific countries. Dr. Flavio was instrumental for establishment of International Collaborating Centre for Aquaculture and Fisheries Sustainability (ICAFIS) and its development into a leading organization in aquaculture and fisheries sustainability. Currently, he is the Director - Aquaculture, Agrochemicals, Vietnam in the Sustainable Trade Initiative (IDH). At IDH, he is now continuing to support the sustainable development of the aquaculture sector through co-financing of sustainability initiatives.

Dr. A.G. Ponniah was Director of the ICAR-NBFGR, Lucknow and ICAR-CIBA, Chennai. He worked as Programme Leader of Biodiversity and Genetic Resources Programme; Discipline Leader of Aquaculture and Genetic Resources, Coordinator of International Network of Genetics and Aquaculture, WorldFish Centre, Penang, Malaysia. He is a recipient of several awards and Fellow of prestigious National

Academy of Agricultural Sciences. He was instrumental in preparation of National Strategic Plan for Aquatic Exotics and Quarantine; Aquatic Exotics and Quarantine Guidelines and risk analysis of Litopenaeus vannamei which is considered as an example of successful introduction of non-native species in Indian waters.

Dr. Chris Hauton is Associate Professor in Invertebrate Ecophysiology and Immune Function within Ocean and Earth Science, National Oceanography Centre Southampton at the University of Southampton. Dr. Chris is a marine ecophysiologist with expertise in assessing how natural and anthropogenic drivers impact the ecophysiology and immunology of marine invertebrates. His research encompasses different levels of biological organization from molecular studies of changes in gene expression to assays of whole organism physiology. He is the Editor for the Invertebrate Survival Journal. He has published > 22 articles and book chapters in peer-reviewed literature and has been awarded ~ £1.3 million in research grant funding from NERC, BBSRC, Royal Society, and the EU since 2001

Prof. Dr. Ir. Pieter van is Chair in Mycology, College of Life Sciences and Medicine, Institute of Medical Sciences, Foresterhill, Aberdeen. He is the Director of the International Centre for Aquaculture Research and Development at the University of Aberdeen. He is also the Microbiology Programme lead in the Institute of Medical Sciences at the University of Aberdeen. He is the President Elect of the British Mycological Society, past Royal Society University Research Fellow, Editorial Board member of Fungal Biology, Editorial Board member of Fungal Biology Reviews. His current research programme focuses on oomycete biology including animal pathogenic oomycetes and plant pathogenic species. He had published more than 100 research papers in high impact journals including Nature.

Prof. Valerie Jane Smith is senior academic at the Scottish Oceans Institute, St. Andrews University, Scotland. She is a specialist in fish and shellfish immunology and pathology and has interest in marine microbiology and biotechnology, particularly regarding disease control in aquaculture and the discovery of new antimicrobials from natural sources. She has established the role of ETosis in marine invertebrates. She has 36 years' experience in academic research and teaching, has supervised 30 Ph.D. students. She is on the Editorial Boards of Development and Comparative Immunology and Fish and Shellfish Immunology, and is a Guest Editor for the journal, Marine Drugs.

Dr. Mansour El Matbouli is Professor and Head of the Clinical Unit for Fish Medicine and Deputy Head of the Clinic in University of Veterinary Medicine, Vienna.

He has expertise in Fish Diseases, Ichthyoparasitology, Fish Bacteriology, Fish Virology and Fish Mycology. He is reviewer for number of scientific journals viz., Anatomia, Histologia, Embryologia, Aquaculture, Diseases of Aquatic Organisms, International Journal for Parasitology, Journal of Fish Biology, Journal of Virological Methods, Veterinary Microbiology, etc. He had published 157 articles and handled more than 16 mega-projects and his Researchgate citation is 3361.

Dr. Eduardo Leano is the Coordinator, Aquatic Animal Health Programme, Network of Aquaculture Centres in Asia-Pacific, Bangkok. He manages the Regional Aquatic Animal Health Programme and provides specialist advice on aquatic animal health matters. He also provides assistance to countries in the Asia-Pacific region on the assessment of health status, institutional development and other activities in strengthening national programmes on aquatic animal health management, disease surveillance and reporting, and other health management measures. He serves as secretary to the Regional Advisory Group on Aquatic Animal Health Management.

Dr. K V Rajendran is working as Principal Scientist and Head, Aquatic Environment and Health Management Division, ICAR-CIFE, Mumbai. He was awarded the Korea Science and Engineering Foundation post-doctoral fellowship and worked at the Yosu National University, South Korea and selected as Fish Pathologist by CSIRO, Australia. He was a Fulbright-Nehru Senior Research Fellow at the Auburn University, USA and worked on pathogen recognition receptors of channel catfish. He has published 45 research papers in international journals. He also acts as peer reviewer of many national and international journals, and national research funding agencies. Overall, Dr. Rajendran has more than 20 years of research experience in the field of fish pathology and crustacean immunology and virology.

Dr. S.K. Sanil is working as a Fish Parasitologist at ICAR-Central Marine Fisheries Research Institute, Kochi. He has about 22 years of research experience in Parasitology & Pathology and 8 years in teaching experience in fish parasitology and pathology at postgraduate level. Presently, he is working in the area of pathogen profiling, diagnostics, and health management in finfish and shellfish including bivalves. He has reported Perkinsus olseni, an OIE-listed disease for the first time in Indian sub-continent from Pinctada fucata and also from a new host i.e. Perna viridis. He also reported infection with Perkinsus beihaiensis in Crassostrea madrasensis from the Indian subcontinent.

Dr. Parimal Roy is the Director, ICAR-National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru. He is product of Madras Veterinary College and worked as Post Doc. Fellow at University of Alabama, Birmingham, Washington State University, USA and Visiting Professor, University of New England and Australia. He worked was Professor in Tamil Nadu Veterinary and Animal Sciences University prior to joining as Director , ICAR-NIVEDI. He is recipient of several prestigious awards including ICAR Jawaharlal Nehru Award; ICAR Rafi Ahmed Kidwai Award and Fellow of National Academy of Sciences and fellow of National Academy of Agricultural Sciences.

Dr. P. K. Sahoo is currently working as a Principal Scientist and ICAR-National Fellow at ICAR-CIFA, Bhubaneswar. He has more than 25 years of research experience in fish disease diagnosis and health management through application of immunological, pathological and molecular tools. He has more than 100 research publications and 2408 citations. He is recipient of several prestigious awards including Jawaharlal Nehru Award for best Ph. D. thesis; Prof. M.S. Swaminathan Best Indian Fisheries Scientist; Professor H.P.C. Shetty Award by Asian Fisheries Society, Indian Branch; Samanta Chandrasekhara Award and Fellow of National Academy of Agricultural Sciences.

Dr. J. K. Jena is the Deputy Director General (Fisheries Science), Indian Council of Agricultural Research, New Delhi. He worked as Scientist, Senior Scientist, ICAR-National Fellow & Head of the Division, ICAR-CIFA, Bhubaneswar from 1992-2010; Director, ICAR-NBFGR, Lucknow during 2010-16. He is recipient of several awards of most of the prestigious societies/agricultural sciences of the country including Special One Time Award of ICAR; ICAR Award for Team Research; Best Young Scientist Award of Dr. Hiralal Choudhuri Fisheries Foundation; Prof. H.P.C. Shetty Award of AFSIB; Pillay Aquaculture Award and Rafi Ahmad Kidwai Award of ICAR. He is the incumbent President of Asian Fisheries Society; Chairman, Asian Fisheries Society Indian Branch. He is the Fellow of prestigious National Academy of Agricultural Sciences. He has been instrumental in development and implementation of the present National Surveillance Programme for Aquatic Animal Diseases.

Prof. Iddya Karunasagar is currently Senior Director (International Relations) at Nitte University, Mangalore. He served as Professor and Head, Fishery Microbiology, Director of Research, Karnataka Veterinary, Animal and Fisheries Sciences University, ICAR National Professor; Senior Fishery Industry Officer, Fisheries and Aquaculture Department, FAO Rome, Italy. He is recipient of several prestigious awards including Professional Fisheries Graduates Forum Dr. M.S. Swaminathan Award; DBT Biotechnology Product and Process Development Award; Academic Contributor of the Biennium Award, International Association of Fish Inspectors, ICAR Rafi Ahmed

Kidwai Award. He is fellow of several prestigious societies including National Academy of Agricultural Sciences.

Prof. C. V. Mohan is the Senior Scientist Aquaculture, WorldFish. He supports the implementation and delivery of the Sustainable Aquaculture Research Program with a focus on Aquatic Animal Health Management. He holds a PhD in Aquatic Animal Pathology from the University of Stirling, Scotland, and worked as an academic for 20 years at the College of Fisheries, University of Agricultural Sciences, Mangalore, India. Before joining WorldFish in April 2014, he worked for the Network of Aquaculture Centres in Asia-Pacific based in Bangkok for 11 years supporting sustainable aquaculture research and development programs with 18 Asia-Pacific governments. He was the Chairperson of the Fish Health Section (FHS) of Asian Fisheries Society (AFS) for the period 2011–2014 and has published over 100 papers in peer-reviewed national and international journals.

Dr. Neeraj Sood is working as a Principal Scientist at ICAR-NBFGR. He is currently the Consortium Principal Investigator of the present 'National Surveillance Programme for Aquatic Animal Diseases' with funding support of £3.5M in which 27 national institutes are participating. He is also Principal Investigator of DBT-BBSRC-DFID project 'Poverty alleviation through prevention and future control of the two major socioeconomically-important diseases in Asian aquaculture'. He has published over 50 publications and his research focus has been on development of monoclonal antibodies against immune cells of commercially important fish species, development of cell lines exhibiting functional characteristics of macrophages and studying innate and adaptive immune response of host against fish pathogens.

Author Index

ISAAHE-102

Survey of Aquamedicines and Formulations Used in Aquaculture

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Survey was conducted to collect information on aquaculture medicines/drugs/chemicals being used in aquaculture in Odisha, Chhattisgarh, Jharkhand and Andhra Pradesh. The information was collected based on questionnaire, in order to develop a database on aquaculture medicines/drugs/ chemicals/ kits etc. being used in aquaculture practices in different regions in India. The data were collected from the fish farmers, dealers, stockiest, shops etc. based on standard format. In Odisha state, information were collected from different districts viz. Angul, Dhenkanal, Cuttack, Khordha, Puri, Ganjam, Balasore, Bhadrak, Nayagarh, Mayurbhanj, Sambalpur, Jajpur, Baragarh, Sundargarh Koraput, Kalahandi, Rayagada and Deogarh etc. In Jharkhand state, survey were made in Ranchi, Bundu, Basai, Barhi, Hazaribagh, Chandil, Patratu and Ramgarh. In Andhra Pradesh, visits were made to East Godavari, West Godavari, Vijayanagaram, Vishakhapatnam and Srikakulum districts. It was observed that around 180 different brands of chemicals/products viz. 25 disinfectant and sanitizer products of aquatic habitat; 42 antimicrobial products, 33 anti-parasitic agent; 24 different probiotic formulations, 32 brands of feed supplements or growth promoters; 8 Oxygen purifier or supplier materials and 16 phytoplankton growth supplements. In Chhattisgarh, it was noticed that there were no established or registered aqua medicine shops but progressive farmers were using different aqua drugs/ chemicals/ products in fish culture by procuring the materials from different suppliers from Andhra Pradesh and West Bengal. In Odisha out of total products being used aquaculture 27% belonged to antimicrobials and anti-parasitic preparations, 20% feed supplements and 7% probiotics category. However, In Andhra Pradesh, maximum products (18%) were feed supplement, growth promoters while (13%) toxic gas removers, pond probiotics (12%), anti-parasitic products (10%) and antimicrobials (9%). The details of survey and availability of products in local markets have been elaborated in the present paper.

ISAAHE-103

Fish Disease Outbreaks and Economic Loss due to Disease in Aquculture

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Aquaculture is the fastest growing food production sector in the world and provides a significant contribution to economic development of rural poor. However, disease has become a primary constraint to aquaculture growth and is now responsible for the severe impact on both the economic and socio-economic development in India. A multitude of factors has contributed to the health problems currently faced by aquaculture. A wide range of pathogens, environmental factors and poor husbandry factors have led to cause heavy losses in aquaculture. In order to understand the prevalence of fish diseases and economic loss caused due to frequent mortality due to infectious and non-infectious factors, survey were carried out at selected regions in Odisha and Andhra Pradesh. Samples were from selected farms and examined for gross pathology and presence of parasites on skin and gills. Detailed information on farm inputs, preventive and control measures taken, culture type, stocking density etc. were collected and correlated with occurrence status. It was observed that occurrence of bacterial disease, followed by parasitic infestation mostly, Argulosis and Gill fluke disease, were responsible for mortality or production loss in the system. Poor management practices, high stocking densities and seasonal climatic change were the important factors responsible for mortality and leading to economic losses to farmers. The details of disease outbreaks in selected farms in Odisha (Puri district) and Andhra radish (West Godavari District) have been elaborated in the present paper.

ISAAHE-104

Modulation of Immuno-haematological Parameters by Heat Killed Whole Cell *Pseudomonas aeruginosa* and its Antagonistic Effect on *Aeromonas hydrophila* in Mrigal, *Cirrhinus mrigala* (Hamilton 1822)

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The application of microbial component for controlling pathogenic bacteria in aquaculture is more promising. The present study showed immunomodulatory effect of heat killed whole cell *Pseudomonas aeruginosa* and antagonistic activity against *Aeromonas hydrophila* in mrigal (*Cirrhinus mrigala*). Total ninety (Control: 45 and Treatment: 45) mrigal (weighting of 40-60g) juveniles was intraperitoneally injected at 1× 106cfu heat killed *P. aeruginosa* per 20 g of body weight. Fishes were challenged against *A. hydrophila* (106cfu/ml) on 14-day post injection and mortality was observed for 7 days. Haematological and serum immune parameters were examined at 1 day before challenge and 6h, 12h, 24h, 48h, 72h, 5day and 7day post challenge. Fishes treated with heat killed P. aeruginosa, showed significantly ($p = 0.05$) enhanced total erythrocyte count, total leucocyte count, NBT, total protein, globulin, bactericidal activity, lysozyme activity, myeloperoxidase level and total anti-protease activity but haemoglobin did not differ significantly ($p > 0.05$) and a non-significant decrease in serum albumin and albumin/globulin ratio was observed in treatment groups compared to control. Treated fishes showed significantly ($p = 0.05$) higher relative percentage survival (44.40%) when compared with the control. This study indicated that heat killed *P. aeruginosa* is a potential immuno-haematological modulator and accelerated survival against *A. hydrophila* infection. Therefore, heat killed whole cell *P. aeruginosa* could be used as potential immunostimulant in aquaculture.

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