Research progress during 2017-18

The centre has taken up projects in are of breading and promoting

(i) indigenous culturable fish species – breeding /RAS system
(ii) Developing low cost feed using locally available ingredients.
(iii) Address the health issues of aquatic animals
(iv) Propagating the culture practices for the overall development of fisheries

Project and Principal Investigators

S.No	Project	Project Leader
1.	Integrated system for aquatic animal health research and management.	Dr. Devika Pillai
2.	Development and standardization of farmer friendly, cost effective techniques for production of live feeds for aquaculture / Establishment of a fish and shrimp seed bank	Dr.Shyama. S
3.	Biotechnological interventions in aquaculture for sustainable production.	Dr. Rejish Kumar V J
4.	Development of sustainable aqua feed from wide spectrum raw materials and propagation of feed based aquaculture in Kerala.	Dr. Chiranjiv Pradhan
5.	Developing brood bank for sustainable production of Karimeen, Etroplus suratensis (Bloch, 1790) and an initiation of selective breeding program	Dr. Binu Varghese
6.	Intensive culture practices for economically important species	Dr. Linoy Libiny

Project Report of CAAHM in Brief

I. Research projects undertaken

- **Isolation and characterization of new potential probiotic bacteria based on quorum sensing system**

- **Molecular characterization of multidrug resistance genes and mechanism of transfer among bacteria associated with freshwater and brackish water aquaculture systems of Kerala**

Principal Investigator- Dr. Devika Pillai, Professor & Head of the Department, Department of Aquatic Animal Health Management

Major Research Outcome

- **Isolation and characterization of new potential probiotic bacteria based on quorum sensing system**

Objectives achieved

- **Isolation and identification of Quorum quenching bacteria from the fish culture pond and gastrointestinal tract of *Oreochromis niloticus***

  Bacteria capable of inhibiting the quorum-sensing signals (Quorum quenching bacteria) were isolated from fish intestine and soil from culture pond. Genus level identification of these bacteria was carried and six isolates showing the presence of auto inducer inactivation gene were selected for further characterization. A probiotic preparation consisting of *Lactobacillus plantarum* QQ8, *Lactobacillus casie* QQ10, *Enterococcus faecium* QQ12 and *Bacillus thuringiensis* QQ17 had excellent quorum quenching and probiotic activities and could prevent infection by pathogenic *Aeromonas hydrophila* in gold fish.

  The multispecies probiotic combination for prevention of disease in C*arassius auratus* was optimized using response surface methodology. This preparation can be administered in feed in aquaculture to control bacterial diseases. Further studies are planned to study the effect of this preparation under field conditions.

- **Molecular characterization of multidrug resistance genes and mechanism of transfer among bacteria associated with freshwater and brackish water aquaculture systems of Kerala**

  Antimicrobial resistance is a major global public health concern and considered as a serious food safety issue. Increasing resistance to antimicrobial agents remains a major challenge in both developed and developing countries. However, in developing countries antimicrobial resistance is impaired by over prescription of antibiotics and increased use in both human and animal healthcare. The bulk amount of antimicrobial use is one of the major reasons in the development and spread of antimicrobial resistance. The 'One health approach' that overuse of antibiotics in one sector can adversely affect the health of animals in other sectors has been realized. The objective of the study undertaken was to screen bacterial isolates from sediment, water and aquatic organisms from water bodies and aqua farms near to selected hospitals. Samples were collected from Thalassery (near to Cooperative hospital), Ernakulam (near to Lakeshore and Aster Medicity) Kollam (Govt. ESIC hospital), Alappuzha (Vandaanam Medical College) and Idukki. Results clearly indicate the occurrence and spread of AMR bacteria of clinical significance in the aquatic environment in the selected areas from hospitals and even to aqua farms in the vicinity where the farmer had not used any antibiotics during culture.
  The Principal Investigator is a part of the team working in KARSAP, the Kerala Antimicrobial Resistance State Action Plan in which medical fraternity, veterinary and fisheries professionals, environment scientists and pharma companies have come together to control the serious issue of rising AMR.

- The Principal Investigator Dr. Devika Pillai is the chairperson of the State Disease Surveillance team.

Establishment of the Laboratories

- Established a cell culture lab in the Dept. of AAHM

A sophisticated cell culture lab has been established in the Centre for Aquatic Animal Health Management that focuses on developing cell line from different tissues of fish. Studies have been initiated. The facility is also used to train the post graduate students specializing in Aquatic Animal Health.

- **Diagnostics services for fish diseases (from March 2018- July 2020)-**
  72 samples (other than samples screened in Surveillance project, which is an NFDB funded network project for disease surveillance in the state)

  Revenue Generation (Testing of samples and training) from March 2018- July 2020 – Rs.2, 20,708/-

- **Advisory services and technical consultancy to farmers & government –**are provided to 150-200 farmers on an average/year

- **Training programme conducted-**

1. **Aquatic Animal Disease diagnosis and preventive management**: **Two** hands- on training programmes of **six days duration** were conducted in the Dept.of AAHM, KUFOS to the state fisheries department officials, as part of the Centre's activity. Twenty officials participated in each of the training programmes.

2. **Biofloc training:** A one day training on biofloc technology was organized for the State Fisheries Department on May 30, 2020. The training consisted of online theory classes followed by a practical session on setting up of a homestead biofloc unit. The online classes were handled by Dr. Krishna R. Salin from Asian Institute of Technology, Thailand; Dr. Babitha Rani, Senior Scientist from Central Institute of Fisheries Education, Mumbai; Dr. Pradeep C., Subject matter specialist, KVK, Kozhikode and Dr. Devika Pillai from KUFOS.

List of Publications from the Project

1. Sneha Kalasseril Girijan, Robin Paul, V.J. Rejish Kumar, **Devika Pillai**. Investigating the impact of hospital antibiotic usage on aquatic environment and aquaculture systems: A molecular study of quinolone resistance in Escherichia coli. (2020). Science of The Total Environment. In Press. Impact factor: 6.5

2. Sneha Girijan Kalasseril, Rahul Krishnan, Rejish Kumar Vattiringal, Robin Paul, Philip Mathew, **Devika Pillai**. Detection of New Delhi Metallo β lactamase 1 and Cephalosporin resistance genes among carbapenem resistant Enterobacteriaceae in water bodies adjacent to hospitals in India. (2020). Current Microbiology, https://doi.org/10.1007/s00284-020-02107-y.

3. Divya V Haridas and **Devika Pillai** (2020). Quorum quenching potential of Enterococcus faecium QQ12 isolated from gastrointestinal tract of *Oreochromis niloticus* and its application as a probiotic for

the control of *Aeromonas hydrophila* infection in gold fish *Carassius auratus* (Linnaeus 1758). Brazilian journal of microbiology. 51 (1): 202-214

4. Divya V Haridas and **Devika Pillai** (2019). Evaluation of quorum quenching and probiotic activity of *Bacillus thuringiensis* QQ17 isolated from fish culture pond. International Journal of Current Microbiology and Applied Sciences, 8(5):1634-1649.
5. Kummari Suresh, Divya V Haridas, Sam Peter, Sevok Handique, Rakesh C.G, Sneha K.G, B.Manojkumar and **Devika Pillai** (2017). Incidence of Heatopancreatic microsporidiasis,by *Enterocytozoon hepatoenai* (EHP) in *Penaeus vannamei* culture in Nellore District,Andrapradesh,India and the role of management in its prevention and transmission. International Journal of Current Microbiology and Applied Sciences. Volume 7 Number 02 .2125-2134

Papers Under review

1. Sneha K.G, Devika Pillai (2020)"Identification andcharacterization of vancomycin resistant *Staphylococcus aureus* (VRSA) harboring vanA and vanB resistance genes in hospital wastewaters in Kerala, India".3 Biotech (Under review)
2. Divya V Haridas and Devika Pillai (2020).Quorum quenching bacteria isolated from nile tilapia intestine protect goldfish from *aeromonas hydrophila* infection. Journal of Microbiology, Biotechnology and Food Science (Under review)

Papers Presented in Conference/Seminars/Workshop

1. Divya.V.Haridas & Devika Pillai 2017. Paper presented on "Probiotic potential of quorum quenching bacteria isolated from fish culture pond and gastrointestinal tract of *Oreochromis niloticus*" in 11th Indian Fisheries & Aquaculture Forum on the topic of "Fostering innovations in Fisheries & Aquaculture, focus on sustainability & safety", November 21-24, 2017 at Cochin, India. Abstract Page No-302

2. Bini C Das, Shaheer P, Diviya V Haridas, Chandhini S, Devika Pillai, Rejish Kumar V. J 2019. Presented a poster on "Isolation and Characterization of Quorum Quenching Bacillus Sp. from the Gastrointestinal Tract of *Lates calcarifer* () in World Brackish water Aquaculture Conference (BRAQCON 2019) conducted on January 22-25, January 2019 at Chennai, India

3. Sneha K.G and Devika Pillai 2019. Paper presented on "Identification of carbapenem and extended-spectrum β-lactamase resistance genes in *Klebsiella pneumoniae* and *Escherichia coli* in public water bodies in Kerala, India: Evidence of horizontal spread of antimicrobial resistance" in AQUABE- 2019, International Conference, 28th-30th November 2019 by KUFOS at Cochin

4. Bini C Das, Nithin Jacob, Divya V Haridas, Arunendhu Ramakrishnan, PH anvar Ali and Devika Pillai 2019. Presented a poster on "Recent EUS outbreak in the flood affected fish farms of Kerala" in AQUABE- 2019, international conference, conducted on 28th-30th November 2019 by KUFOS at Cochin

5. Divya V Haridas, and Devika Pillai 2019. Presented a poster on "N-acylhomoserine lactone-degrading Bacillus strains isolated from fish culture pond soil" in AQUABE- 2019, international conference, conducted on 28th-30th November 2019 by KUFOS at Cochin

6. Divya V Haridas, and Devika Pillai 2020. Presented a Paper on "Characterization of quorum quenching soil bacteria Bacillus thuringiensis QQ17 and evaluation of its probiotic activity in *Carassious auratus*" in Kerala Science Congress- 2020, 25th-27th January 2020 by KSCSTE at Palakkad

7. Sneha K.G and Devika Pillai 2020. Paper presented on "Identification of multidrug resistant mecA gene positive *Staphylococcus aureus* and *Acinetobacter baumannii* isolates in aquatic environment". in Kerala Science Congress- 2020, conducted on 25th-27th January 2020 by KSCSTE at Palakkad.

II. Research projects undertaken

Title: Establishment of Fish and Prawn Seed Bank

Principal Investigator:Dr.Shyama.S,

Professor

Department of Aquaculture

Major Research Outcome

Work done in relation to the envisaged Objectives

a. Dietary enhancement of reproductive potential

- Progress of Ornamental Fish Culture is severely constrained by the paucity of quality seed and very low larval survivallevels.
- Dietary approach is a key avenue for addressing these issues. A variety of ingredients were screened with respect to their utilityin brood stock diets for ornamentals, for enhancing the reproductive potential of ornamentals.
- The range of ingredients screened included sources of plant origin (13), processing wastes (6) and animal origin (6).Screened ingredients were employed in formulation of diets for brood stock of different ornamental species, to assess their influence on reproductive potential, in different ornamentals.
- Trials, incorporating the ingredients assessed, for formulation and standardizationof brood stock diets, employing the more promising sources, are in the second phase of evaluation.
- Inclusion of ensiled / fermented forms of the ingredients, gave better breeding response than the base forms
- Processing wastes, principally fishery processing wastes, were found to be a cost-effective and efficient source in improving reproductive response in ornamentals
- Ingredients with significant carotenoid content, led to enhanced fecundity and better larval survival

- Both Live bearing and egg laying varieties of ornamentals were taken for the studies
- Stock of different ornamentals (17 varieties) were maintained for conducting studies

b.Enhancement of coloration of ornamentals

- Colour and variety are the factors deciding the market value of ornamentals.
- Ingredients of both plant and animal origin were assessed for their carotenoid content
- Base ingredients as well as ensiled / fermented forms were evaluated by inclusion in formulated diets
- Evaluation of the colour enhancement potential in ornamentals, was done over a 90 day study period, for each individual ingredient
- Preliminary trials on the use of plant ingredients in combination with animal source ingredients, gave encouraging results with respect to colouration
- Further trials for development of colour enhancement diets along the above line, is being continued
- Formulation of colour enhancement diets is being linked with processing waste management

Presentations made

1. AswathyBabu, Shyama. S,NeethuNorman andAryasree P.V. 2019.Sour paste nematodes as live feeds – an appraisal. Poster presentation in AQUABE, 2019, International Conference on Aquatic resources and Blue economy, 28-30 November , 2019 , Le Meridien , Kochi , organized by Kerala University of Fisheries and Ocean Studies, Panangad,Kochi. Abstract No. APS – PO -23, pg.no.94. Book of Abstracts

2. Shyama.S,AswathiBabu ,Aryasree P.V. andNeethu Norman. 2019 .Evaluation of different substrates for raising *Moinamacrocopa* . Poster presentation in AQUABE, 2019, International Conference on Aquatic resources and Blue economy, 28-30 November , 2019 , Le Meridien , Kochi , organized by Kerala University of Fisheries and Ocean Studies, Panangad,Kochi. Abstract No. APS – PO -18, pg.no. 90. Book of Abstracts

3. Aryasree .P.V, Shyama. S, AswathyBabu, and Neethu Norman. 2019. Comparative assessment of the production of *Moinamacrocopa* at different stocking densities. Poster presentation in AQUABE, 2019, International Conference on Aquatic resources and Blue economy, 28-30 November , 2019 , Le Meridien , Kochi , organized by Kerala University of Fisheries and Ocean Studies, Panangad,Kochi. Abstract No. APS – PO -19, pg.no. 90, Book of Abstracts.

4. Neethu Norman, ShyamaS, Aryasree P.V. andAswathiBabu. 2019.Life history parameters of Moinamacrocopa raised in different media. Poster presentation in AQUABE, 2019, International Conference on Aquatic resources and Blue economy, 28-30 November , 2019 , Le Meridien , Kochi , organized by Kerala University of Fisheries and Ocean Studies, Panangad,Kochi. Abstract No. APS – PO - 20, pg.no. 91, Book of Abstracts

5..AswathyBabu, Shyama.S, Neethu Norman, Aryasree P.V, and Yash Sunil Pawar . 2019 Live feeds in ornamental fish culture – an appraisal. Poster presentation in 9th International

Science Congress, ISC-2019, ISCA , December 8-9 , 2019, Abstract No. ISCA-ISC-2019 - 2AVFM-04 –Poster, pg.no. 91 Book of abstracts

III. Research projects undertaken

Development of sustainable aqua feed from wide spectrum raw materials and propagation of feed based aquaculture in Kerala

PI.Dr.Chiranjiv Pradhan, Assistant Professor, Department of Aquaculture

Objectives achieved

- **Evaluation of cashew nut processing waste (CPW) as a nonconventional feed ingredient in the diet of tilapia, *Oreochromismossambicus***

It is demonstrated that 50% SBM can be replaced with CNM without compromising growth, nutrient utilization, body composition and health status of tilapia. CNM is locally available in a much cheaper price than SBM. CNM appears to be a suitable ingredient in tilapia feed at 40% incorporation level along with 20% soybean meal and resulted in better fish performance. In addition, CNM 4 also exhibited the lowest economic conversion ratio of cost INR 63.28 (USD 0.88) among the experimental diets followed by CNM 3, CNM 2, CNM1, and CNM 0. However, more study is required to ascertain the optimum level of replacement of SBM with CNM in pond production trials of longer duration.

- **Effect of Cassia roxburghii as a Phytogenic Feed Additive on Growth Performance, Nutrient utilisation and Health status of Oreochromismossambicus**

It can be concluded from the study that supplemented of cassia roxburghii bark extract (1%) in the diet is suitable for higher growth, nutrient utilization and health status of Oreochromismossambicus

- **Dietary taurine requirement of *Pangasidonhypophthalmus* fry, based on growth, nutrient utilization, digestive and antioxidant enzyme activities**

Results of the current investigation revealed the essentiality of the dietary taurine supplementation for maximizing growth, body composition, digestive enzyme activity and antioxidant activities of pangasius fry. Broken line regression analysis of weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio against taurine concentrations indicated the taurine requirements at 15.03. 15.33, 15.17, 16.31 g/kg, respectively. On the basis of results obtained in this study, dietary taurine requirement in the range of 15.03 to 16.31 g/kg is recommended for Pangasidonhypopthalmus.

- **Effect of fishmeal replaced diet by yeast fermented guar and copra meal mixture on growth performance, digestibility, digestive enzyme activity, blood metabolites and antioxidant capacity in tilapia, *Oreochromisniloticus***

The results of the present study indicate that fermentation of guar and copra meal with yeast *S. cereviceae* increased the overall nutrient quality by increasing amino acid content and reducing antinutritional factors. The fermented guar and copra meal mixture gave a better amino acid profile suitable for tilapia nutrition. But the replacement of FM more than 25% did not result in higher growth and nutrient utilisation in tilapia. The increase in quantity of

antinutritional factors by the replacement of FM with FGCM mixture attributed to reduced digestive enzymes activity and nutrient digestibility in fish. The antioxidant enzymes and blood parameters were also affected by the incorporation FGCM mixture by replacing FM. The cubic regression of the result showed that FGCM mixture can replace fishmeal from 16.38 to 23.94 % in the feed of tilapia for better growth and body composition.

- **Effect of dietary L-carnitine/α-ketoglutarate ratio on growth performance, digestive enzymes, hematological characteristics, antioxidant capacity and innate immunity in tilapia, *Oreochromisniloticus***

In this present experiment, the better fish performance also exhibited while fed by higher LC proportion in the diet. However, the regression analysis of the results showed that the suitable ratio of LC/AKG for better fish performance was between 5 to7 which indicated that the contribution of both LC and AKG. It can be concluded from the study that there is a positive effect of the higher ratio of LC/AKG on growth performance, nutrient utilisation, digestive enzymes and health of tilapia, Oreochromisniloticus.

Establishment of the Laboratories

Established two laboratories in the Department of Aquaculture

1. Wet laboratory for feeding experiment

2. Feed evaluation laboratory

Awards

Won Best Poster Award

Chiranjiv Pradhan was awarded certificate for best poster presentation for the presentation of research paper entitled "Replacement of soybean meal with Cashew Nut Meal (CNM) as an alternative protein source in the diet of tilapia, *Oreochromismossambicus"* during the BRAQCON 2019,World Brackishwater Aquaculture conference, organised by the society of Coastal Aquaculture and Fisheries (SCAFi) and ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA) at Chennai during 23-25 January 2019.

List of publications from the project

1. Pradhan C., Gopinath, D.B., Namitha D., Nikhila P., Sankar, T.V (2020). Replacement of soybean meal with cashew nut meal as an alternative protein source in the diet of tilapia, Oreochromismossambicus. Aquaculture Research. Doi: 10.1111/are.14512.

Papers Under review

1. NamithaDileep, Chiranjiv Pradhan, Nikhila Peter, Dinesh Kaippilly, T.V. Sankar (2020). Effect of fishmeal replaced diet by yeast fermented guar and copra meal mixture on growth performance, digestibility, digestive enzyme activity, blood

metabolites and antioxidant capacity in tilapia, Oreochromisniloticus. Aquaculture Nutrition (Under review)

2. Chiranjiv Pradhan, UzmaSoharwardi, NamithaDileep, Nikhila Peter, Rachel Fernandez, AmithaKurian, PreethamElumalai (2020). Effect of dietary L-carnitine/α-ketoglutarate ratio on growth performance, digestive enzymes, hematological characteristics, antioxidant capacity and innate immunity in tilapia, *Oreochromisniloticus.* Animal feed science and technology (Under review)

3. Nikhila Peter, Chiranjiv Pradhan,NamithaDileep, Mohammad Musharraf, T.V. Sankar (2020). Effect of dietary taurine on growth performance, nutrient utilization, digestive and antioxidant enzyme activities in *Pangasidonhypophthalmus* fry. Journal of world aquaculture society. (Under review)

Some other publications

4. Pradhan C., Giri, S. S., Mohanty, T. K., Mohanty, S. N (2020). Feeding a diet containing plant ingredients at different levels influenced growth performance, carcass composition and blood biochemical parameters in Indian major carps grown in polyculture earthen ponds. Tropical Animal Health and Production. 52, 1769–1777. doi:10.1007/s11250-019-02184-7.
5. Pradhan C., Das, D.R., Mohanty, T. K., Giri, S. S. (2020). Evaluation of nutritive value, fatty acid composition and lipid quality indices of freshwater catfishes and murrels. Journal of Aquaculture and Marine Biology. 9 (3), 80-85.
6. Pradhan, C., Giri, S.S., Mohanty, S.N., Rajendran, N. (2019). Effect of different feeding levels of plant ingredient based feed on fillet fatty acid profile, carcass trait and sensory characteristics of Indian major carps reared in polyculture ponds. Journal of World Aquaculture Society. 50(2): 374-389

Popular article published in Magazines

1. Chiranjiv Pradhan and AbhilashSasidharan(2020). Sensory characteristics: a novel way of evaluation of food fishes. Science Magazine "Sasthra" (Communicated)

Papers Presented in Conference/Seminars/Workshop

1. Namitha D., Nikhila P., Pradhan, C. (2019). Replacement of fishmeal with a mixture of fermented guar and copra meal in the diet of genetically improved farmed tilapia (GIFT). International conference on aquatic resources and blue economy, Kochi, Kerala, 28-30 November. pp 96
2. Nikhila P., Namitha D., Pradhan, C. (2019). Quantitative taurine requirement for Pangasius hypophthalmus fed casein based diet. International conference on aquatic resources and blue economy, Kochi, Kerala, 28-30 November. pp68
3. Dinesh, K., NilimaPriyadarshini, PankajGargotra Pradhan C. (2019). Significance of Guar (Cyamopsistetragonaloba) in aquafeed industry towards replacing fishmeal: merits and demerits through practical lessons. Fish Feed and Nutrition workshop organized by School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Auburn Al, United States Of America, 10-20 September
4. Nilima, P., Dinesh, K., Pradhan, C. (2019). Effect of guar sprout meal on the growth and nutrient utilization of tilapia fingerlings Oreochromisniloticus. Asian-Pacific aquaculture conference, Chennai, India, 19-21 June.
5. Nithin, J., Namitha, D., Nikhila, P., Pillai, D., Pradhan, C. (2019). Effect of Cassia roxburghii as a plant-based feed additive on growth performance, nutrient

utilisation and health status of Oreochromismossambicus. World Brackishwater Aquaculture conference (BRAQCON), CIBA, Chennai, 23-25 January.pp 143.

6. Balagopinath, D., Namitha, D., Nikhila, P., Pradhan, C., (2019). Replacement of soybean meal with Cashew Nut Meal (CNM) as an alternative protein source in the diet of tilapia, Oreochromismossambicus. World Brackishwater Aquaculture conference (BRAQCON), CIBA, Chennai, 23-25 January.pp 149.

Book

- Pradhan, C., Rachel F., Namitha D., Nikhila P. 2019. Farmers hand book on "Farm made feed and feeding of some important cultured fish species of Kerala". Kerala University of Fisheries and Ocean Studies

Training programme conducted

- Conducted training programme on "Farm made feed preaparation" in Payannur substation, KUFOS on 12 March 2019 and published booklet entitled farm made feed for the culture of carp, tilapia and pangasius. (70 famers participated)

Consultancy

I have expressed my willing ness to support some progressive farmers in establishing fish feed mill and formulation of fish feed.

IV. Research projects undertaken

Developing brood bank for sustainable production of Karimeen, Etroplus suratensis (Bloch, 1790) and an initiation of selective breeding program

PI: Dr. Binu Varghese, Assistant Professor, Department of Aquaculture

Objectives achieved

- Karimeen seeds were supplied to farmers in the nearby districts for farming,

Karimeen seeds were given to research institutions, college researchers and students for various studies(beneficiaries include KUFOS, CUSAT, CIFT and nearby colleges)

- Five MFSc students did their research on Karimeen seeds and brooders of the project,
    1. Broodstock nutrition studies
    2. Effect of salinity on growth and survival during seed production.
    3. Monosex seed production using hormone
    4. Use of Cladoceran livefeeds in larval rearing
    5. Use of Haematococcus enrichment on larval rearing

- Brooders were given to three farmers to produce seeds with advice on maintenance and breeding and larval rearing.
- Live feeds like microalgae, moina, daphnia, microworms etc supplied to farmers.

- Facilities developed for the project were used for giving practical training to UG and PG students as part of curriculum.
- Participants of Earn While You Learn programme (for B.F.Sc students) were given exposure to seed production and tank and pond culture of Karimeen.

- Two laboratories established as part of the project and equipment procured.
    1. **Fish Genetics and Breeding Laboratory**
    2. **Microalgal Laboratory**

- Recirculatory larval rearing systems were developed
- Developed system for mass production of Karimeen seeds (scale up model)
- Guided visit was provided to Hatchery in-charge/technicians of Department of Fisheries from various districts. Basic operation protocols for the breeding, artificial incubation and larval rearing was imparted.

- Handbook of pearlspot seed production prepared and malayalam translation and printing is progressing. Which will be made available to farmers.
- Results of the research was presented in AQUABE conference.
- Webinars were conducted on Karimeen farming and seed production during lockdown period
- Farmers were given advice on Karimeen culture and breeding.

V. Research projects undertaken

Intensive culture practices for economically important species

PI: Dr.LinoyLibini, C. Assistant Professor, Department of Aquaculture

Major Research Outcome

Objectives achieved

- **Rearing of mud crab in individual cellular rearing system (ICRS) for improved production and survival**

The growth and survival of the crab was analyzed in different weaning system adopted with difference in enclosure and feed type namely treatment I, II, III & IV. The FCR values were showed a better performance in all treatments, in which self-formulated feeds (Pellet feed and flakes feed) were trialed than that of the control (fed with commercial shrimp pellet feed). Among the treatments, the best feed conversion ratio was recorded is 2.2.

- **Culture trails with formulated feeds - promote and recommend such feeds for farming practices**

A rearing feed is formulated and prepared with hand pellatizer with locally available ingredients for weaning. The prepared feed was offered a crude protein content of 41.48%, crude lipid of 8.10%, Crude fibre of 4.06%, nitrogen free extract of 25.98% and ash content of 12.49% with almost 8% moisture.The total cost was estimated as approximately Rs. 112 per kg of feed and bulk purchase and commercial scale feed production using advance machineries technology will able reduce the cost upto 20-25 %.

- **Culture of *Etroplus*suratensis using Recirculating aquaculture systems (RAS)**

The fish pearlspot (*Etroplussuratensis*) known as "Karimeen" in Kerala, is an excellent table fish of delicacy and considered as state fish have good market value. The culture of pearl spot in RAS showed relatively moderate growth rate. But, the fish have attained a marketable size of 120-150 g over a period of 8 months. Though growth rate is relatively slow, high stocking density with low input management can yield optimum production.

- **Utlility of seaweeds in nutrients conversion and culture them in biofilteration systems in RAS**

Studied the utlility of seaweeds such as *Caulerpa taxifolia, C. Scalpelliformis* and *Dictyota dichotoma* in nutrients conversion and culture them in biofilteration systems.

Seaweeds remove the nutrients which is adversely affecting the water quality parameters can be utilized to convert in to biomass which is highly useful for extraction for its biochemical contents. With based on RAS and similar aquaculture production systems, seaweeds can utilize for bioremediation for improving water quality support more production in fed aquaculture systems Thus commercially important seaweed can be applied to the mariculture activities for water quality management and also to generate income both from fish as well as seaweed.

List of Publications from the Project

Papers Presented in Conference/Seminars/Workshop

1. Chandrabhushan S. Nishad and LinoyLibini, C 2019. Assessment of growth performance of mud crab, *Scylla serrata*on formulated feed.presented in International Conerence on Blue Economy and Aquatic Resources (AQUABE 2019) held at Le Meridian, Kochi on 28-30 November 2019,

2. Viji, C.S., Bimal, P., Shara, A.S., HusnaHaneef, LinoyLibini, C. and T.V. Sankar 2019. Nursery rearing of *Penaeusmonodon*(Fabricius) and *Litopenaeusvannamei*(Boone) in the recirculatory aquaculture system. presented in International Conerence on Blue Economy and Aquatic Resources (AQUABE 2019) held at Le Meridian, Kochi on 28-30 November 2019,

3. Hayin Tamut*, C.and LinoyLibini, C. 2019.**Assessment on bioremediation capacity of seaweeds in recirculatory aquaculture system.** presented in 3rd International Symposium on Marine Ecosystems Challenges and Opportunities (MECOS3),at Central Marine Fisheries Research Institute, Kochi on 7-10 January 2020.

1. consultancies taken up, : Nil

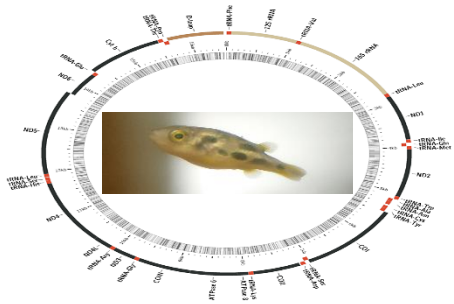
2. support provided to state fisheries dept for taking up innovative projects : Nil

Research achievements

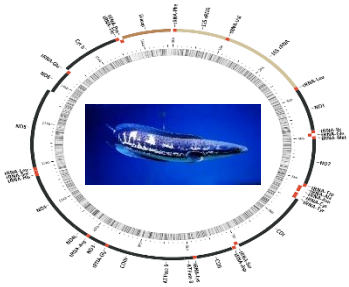
Whole mitochondrial DNA sequence analysis of endemic fishes of kerala:

Fish exhibit amazing diversity in terms of size, shape colour besides many other morphological characteristics and the scientific identification of fish species is not restricted to taxonomists and systematics alone but is also concerned with fishery ecology, fishery management, estimations of recruitment and spawn areas, and authentication of food products. Conventional methods of fish identification often poses problem to scientists because of high diversity and morphological flexibility. Also many times it is difficult to identify them in their diverse developmental stages by using morphological characteristics alone The DNA-based identification techniques, of late, prove to be effective analytical techniques and DNA barcoding has been largely advocated for identification of biological diversity. KUFOS has been using DNA technique to identify selected farmed species and is continued over the years.

- The mitochondrial genome has many properties that make it useful for reconstructing recent phylogenetic history. The sequencing of whole mitochondrial genome of fishes, which are endemic to kerala, viz., *Channa diplogramma, Clarias dussumieri, Horabagrus nigricollaris, Carinotetraodon travancoricus* and *Tor malabaricus has been completed.*

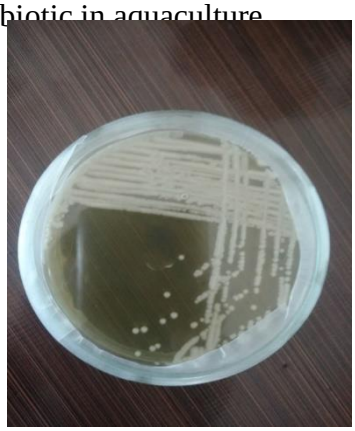


Complete mitogenome of malabar puffer fish *Carinoteteraodon travancoricus*



Complete mitogenome of malabar snakehead *Channa diplogrammae*

- Isolated yeasts from the gut of Tilapia, Etroplus and Seabass for assessing the potential as probiotic in aquaculture



Development of feed from local ingredients

Aquaculture requires optimization of nutrition to efficiently raise fish for the purpose of food production. Fish nutrition is the study of nutrients and energy sources essential for fish health, growth and reproduction. Sustainability of the aquaculture industry is an environmental,

economic and social concern and certain issues related to fish nutrition namely feed and nutrient efficiency, overfeeding and waste, unsustainable feed ingredients, fish health issues, biotechnology and human health concerns have become environmental concern and achieving a balance between efficient and safe food production with environmental sustainability has become a challenge for the industry. It is also clear that more 50% of the cost of production goes towards good feed and is an important problem to be addressed. KUFOS has initiated research in identifying eco-friendly and effective raw materials as replacements for conventional raw materials and some of the initiatives are identified.

Three feeding experiments were intiated to study the effect of natural components on growth of fish viz., Cashew nut processing waste, Dietary Taurine etc. on th performance, nutrient utilization and health status of aquacultured fishes.

A state of art wet laboratory for nutrient digestibility study with re-circulatory facility is ready for feeding experiments

Captive breeding for Etroplus / Recirculatory Aquaculture system

Re-circulatory Aquaculture system (RAS) is recognised as a high-producing and low-risk culture practise to achieve sustainable development of aquaculture. Compared to traditional aquaculture system this has been identified as safe, healthy and cost effective besides being environment friendly.

KUFOS has developed successfully a novel captive breeding setup for Etroplus and successfully bred Karimeen inside the hatchery in FRP tank.Broodstock development and pairing achieved **under** captivity. A Livefeed cultures established for Karimeen. The recirculation systems for brooders and larvae are getting installed.

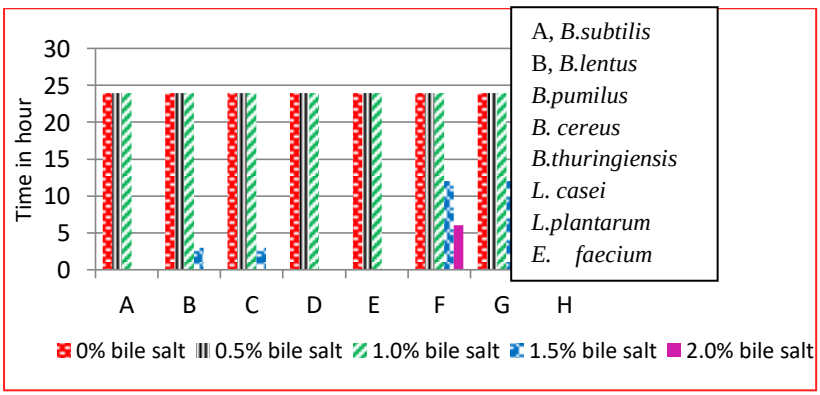
RAS systemfor Etroplus **Etroplus captive breeding**

- Meat quality and nutritional profile of pearlspot from different sality consitions revealed variation in the composition. The amino acid and fatty acid profile of the samples are being done for getting a comprehensive understand on fish quality.

Quorum quenching bacteria as probiotic

The process by which microorganisms monitor and regulate their population density through chemical signaling is termed quorum sensing and the process by which quorum sensing can be inhibited is called quorum quenching. Work over the past few years has confirmed that quorum-quenching mechanisms are widely preserved in many prokaryotic and eukaryotic organisms. These naturally occurring quorum-quenching mechanisms appear to play important roles in microbeemicrobe andpathogenehost interactions.

Diseases constitute one of the most important economic constraints for the development of aquaculture sector. The potential probiotic traits of bacteria as a suitable biocontrol agent in aquaculture were tried. Different strains of QQ bacteria were isolated from gastrointestinal tract of Oreochromis niloticus and soil from fish culture pond and about few showed strong quorum quenching ability. The quorum quenching ability of these isolates was confirmed by the presence of aiiA [Autoinducer inactivation] homologue gene. The bacteria were screened by in vivo experiments for their probiotic activity.



Bile-salt tolerance (0-2%) of isolated strains at different time periods of incubation at 37°C.

AHL degradation assay on LB agar. In each plate, CV31532 (AHL producing positive strain) (right), QQ bacteria (AHL degrading strain)(middle) and CV026 (Bio indicator strain)(left).The area near QQ bacteria (Middle line) of CV026 has no purple pigment indicating the ability of the isolates to degrade AHL molecules. A. *Bacillus thuringiensis*, B. *Enterococcus faecium* C.*Lactobacillus plantarum*, D. *Lactobacillus casei*

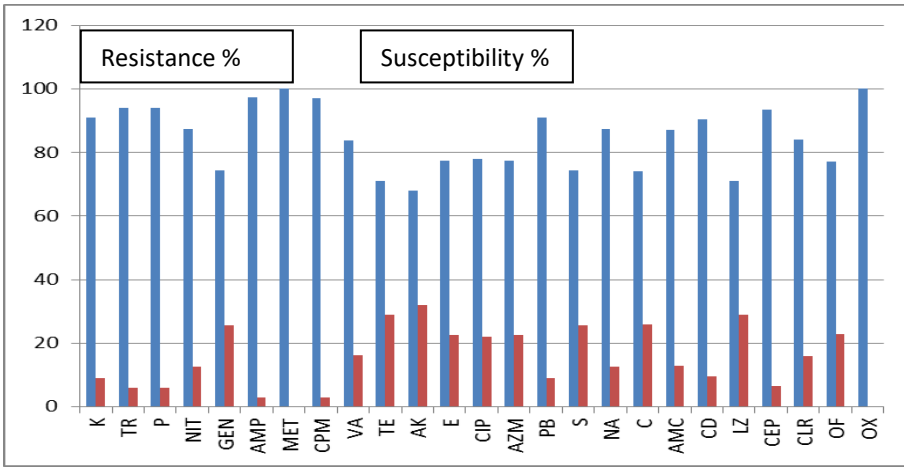
In vivo study with probiotic diet

Surveillance of Antimicrobial Resistance in selected aquaculture farms

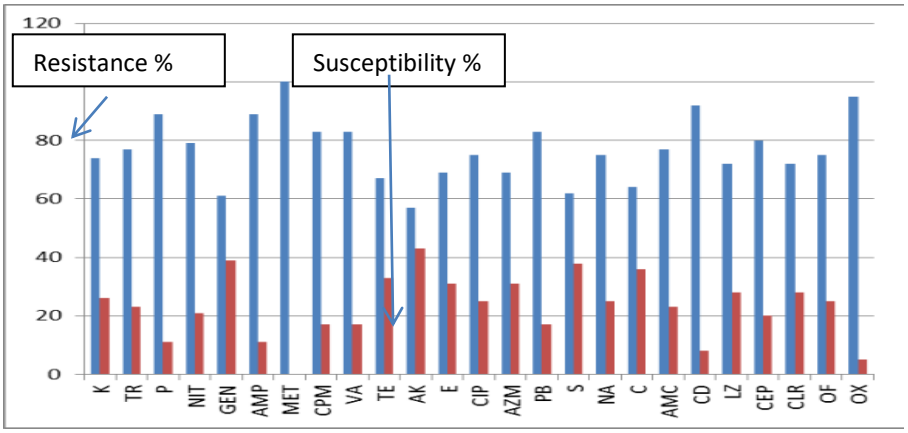
Antimicrobial resistance is a major global public health concern and considered as a serious food safety issue. Antimicrobial agents play a most relevant role in the therapy of bacterial infections in humans, veterinary and aquaculture. The indiscriminate use of antimicrobial agents is one of the major reasons in the development and spread of antimicrobial resistance.

Samples included water, soil, clam, fish, shrimps and crabs from direct discharge from the hospital to public water bodies and places using the same water for aquaculture. All the bacterial isolates were characterized by biochemical methods and a total of 214 isolates were collected from different samples. Most of the isolates belonged to Gram negative *Enterobacteriaceae* family.

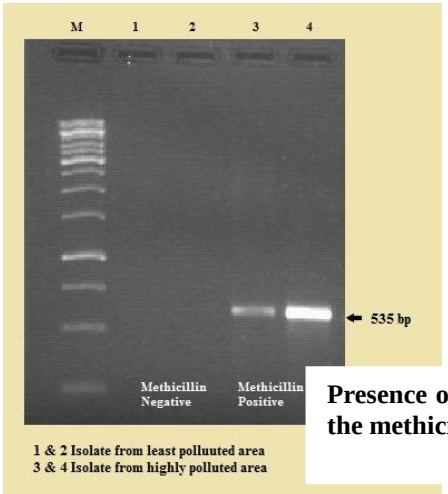
Multiple Antibiotic Resistance (MAR) and Antibiotic resistance Index (ARI) of these isolates were very high. The study indicated that mechanism of transfer of antimicrobial resistance is mainly plasmid mediated.



Antibiotic resistance percentage among bacterial isolates from direct hospital discharge



Antibiotic resistance percentage among bacterial isolates from aquaculture farms located in the vicinity of hospitals



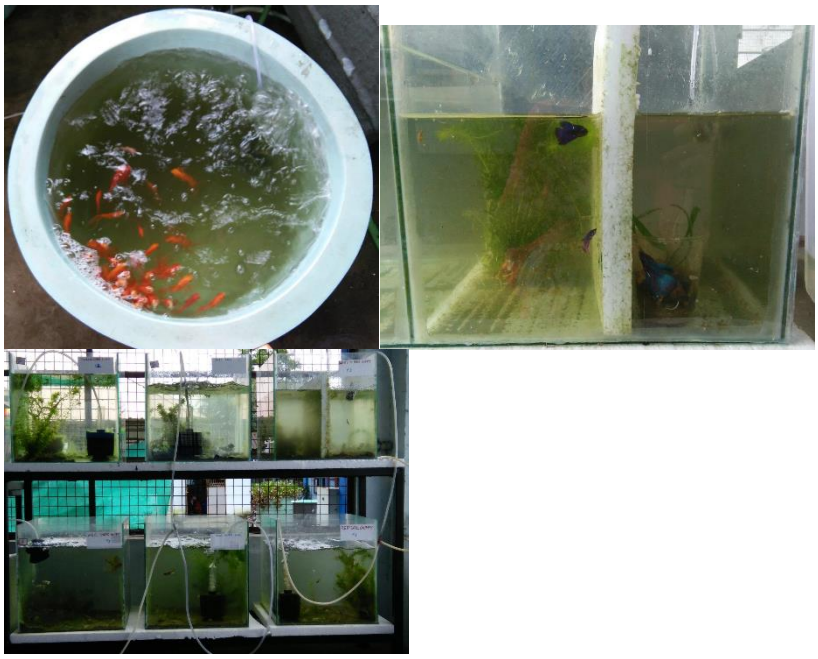
Presence of methicillin resistance gene in isolates Arrow points to the methicillin resistance gene (535 bp

Establishment of fish and prawn seed bank

In order to address the issue of ready and timely availability of research effort has been made on the formulation and evaluation of brood stock diets and improving larval survival of seed fish. While accomplishing the objectives seed production of seven types of ornamental fishes

was carried out *viz*., guppy (seven varieties), molly (two varieties), platy (two varieties) sword tail, Siamese fighter fish and gold fish. The quality brood stock is being raised for sword tail, platy, guppy and Siamese fighter.

Similarly brood stock diets are being formulated **incorporating** processing waste /sprouted grains / indigenous carotenoid sources. A range of locally available, indigenous sources of carotenoids were screened and incorporated in diets for assessing their potential in enhancing the colouration of ornamental fish.



Seed production from gold fish, fighters and Aquarium vertical setup for seed production

Development and standardization of farmer friendly cost effective techniques for production of live feeds for aquaculture

The production of phytoplankton and zooplankton is essential in various industrial activities including aquaculture and aquaculture depends on planktons as a natural food source for the larval stages of many aquacultured species. The use of live feed in larval rearing therefore remains a necessity in most aquatic organism hatcheries. In fact, most early stages of fish larvae do not react to dry feeds and require live feeds that swim actively and stimulate their raptorial behaviour. This is also due to the inability of the fish larvae to digest and assimilate the formulated dry feed. Work has been initiated to determine the culture requirements of different live feeds and preliminary studies, to evaluate different media / substrates for the culture of live feeds and to produce live feeds on mass scale. The live feed organisms taken up for study include moina, microworm, grindal worm, copepods and infusoria. Different low cost and effective culture media and substrates have been assessed for their potential to support biomass production based on the outcome in the above studies, mass culture trials are in progress,

Mass culture of Moina in circular tubs, Paramecium culture using milk and plant materials

Research progress during 2018-19

1. **Developing brood bank for sustainable production of Karimeen, Etroplus suratensis (Bloch, 1790) and an initiation of selective breeding program**

The research on pearl spot progressed in line with the previous years focused area and new initiatives on improving the seed production. Apart from the stocks available from Panangad (Ernakulam), Edathua (Alapuzha) and Azhikode (Thrissur), new stocks were included from Udayapuram (Kottayam). Currently all the four stocks are breeding under captivity and the Alappuzha stock F1 generation started spawning and shortly we will get F2 generation. The innovative outdoor tank setup for continuous mass breeding was successful and are now producing about 10-15 spawning per month and this will be increased to morethan 50 in coming months.

Important Findings:

1) Production of monosex etroplus (all male) (>90%)
2) Broodstock nutrition for optimal growth.
3) Successfully developed system for external incubation for continuous spawning curtailing parental care. Experiments are on for confirming the results..
4) Optimization stocking density and growth of karimeen larvae in freshwater RAS.
5) Mass production and larvae rearing of karimeen in outdoor cement tanks.
6) Maintenance of stock and mass culture of microalgae, moina, rotifers, microworms etc

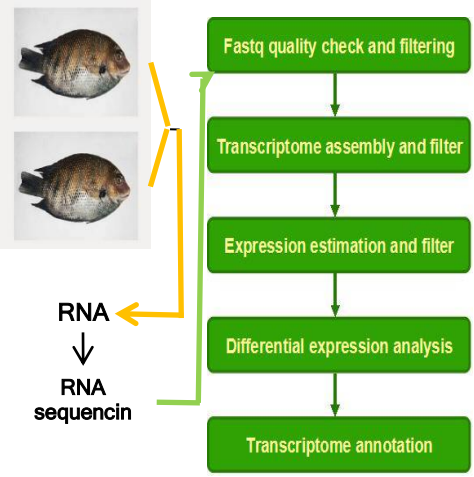
Genetic analysis of different strains and generations of Karimeen, early stage sex identification of karimeen and producing all male pearlspot are some of the envisaged activities.

2. **Transcriptome analysis of *Etroplus suratensis***

Etroplus suratensis is a high demand palatable economic species endemic to Southern India and Sri Lanka. Exploring the biology of the fish at genomic level is important for the enhancement of its productivity.

This is the first in depth transcriptome analysis of *E. suratensis*. Around 96,646 unigenes were assembled *de novo*. The genetic makeup was most similar to *O. niloticus*. Growth factor proteins including those of Fibroblast Growth Factor signaling pathway were found

highly expressed in the female sample among the DEGs. A total of 29,999 SSRs were identified.

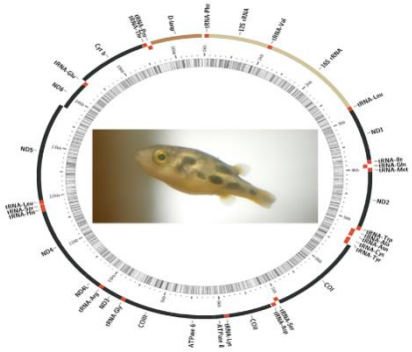


SSR results	Numbers
Total No. of sequence examined	96,646
Total No. of identified SSRs:	29,999
Number of SSR containing sequences:	18,165

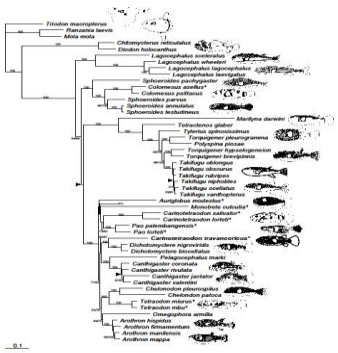
Processes	Major genes
Growth	*fgf,fgfr,igf1&2. gh, megf10*
Reproduction & Development:	*vg5,vgr,zp3,sox8a,dmrt3,zarl1*
Immunity	*TLR5, CISH, A2M,IL17R*

3. Development of molecular markers for the conservation of fishes endemic to Kerala

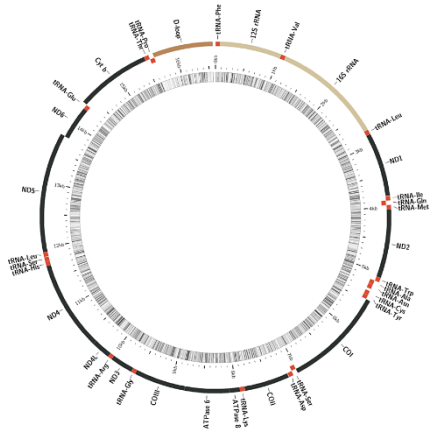
Completed whole mitochondrial genome sequencing, genome annotation and phylogenetic and comparative analysis of *Carinotetraodon travancoricus, Channa diplogramma* and *Horabagrus nigricollaris.*



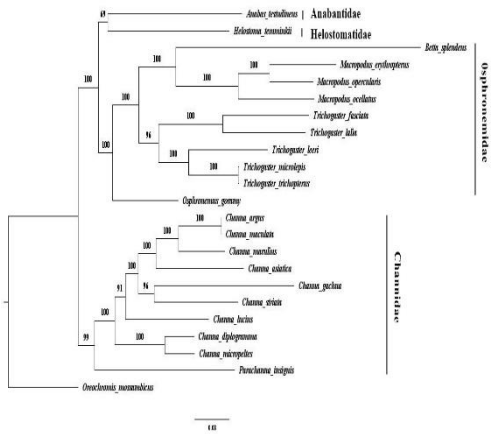
Carinotetraodon travancoricus



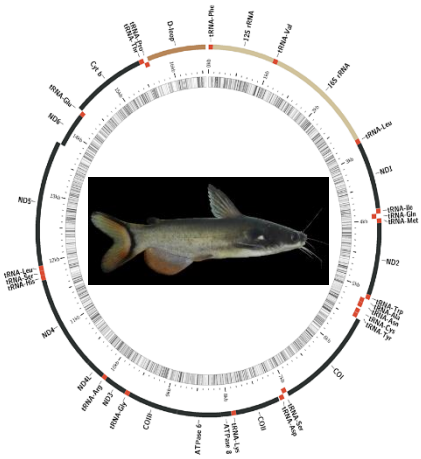
Maximum likelihood (ML) tree using the $12_n3_rRT_n$ data set. Arrowheads indicate the difference from the tree from the 123_nRT_n data sets. The numbers near internal branches indicate bootstrap probabilities of $12_n3_rRT_n$(left) and 123_nRT_n(right) data sets, respectively (values less than 50% not shown). Single numbers indicate that ML analyses for the $12_n3_rRT_n$ and 123_nRT_n data sets resulted in identical values. Asterisks with species names indicate freshwater species.



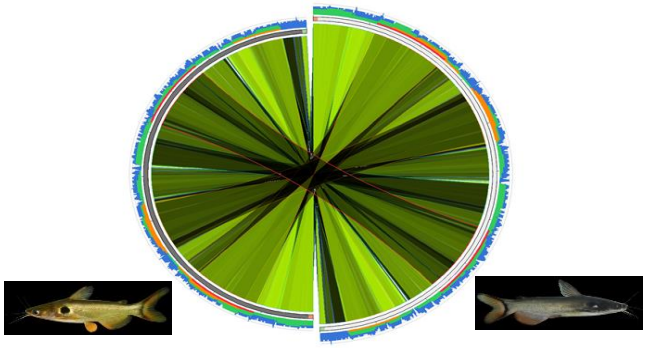
C. diplogramma



Phylogenetic analysis of *C. diplogramma* among the Anabantiformes based on the maximum likelihood (ML) method of the 13 mitochondrial protein coding genes with bootstrap probabilities obtained from 1000 replications (bootstrap values which are more than 50% are shown on the branches)



H. nigricollaris



Structural comparison of *H. nigricollaris* and mitogenome *H. brachysoma*. Green and red block in the beginning and end of the sequences indicate the sequence orientation. Normal sequences are coloured by black and inverted repeats are marked with lime colour. The coloured blocks outside the sequence describe the blast hit scoring, best quartile being red, then orange, green and blue, respectively. The mitogenome of *H. nigricollaris* has 97.6% sequence similarity to that of *H. brachysoma* mitogenome.

4. Culture of Live feeds

Studies are going on the production of live feeds of importance namely Microworm / breadworm, Grindal worm, Infusoria, Paramecium, Zooplankton-cladocerans, copepods and Chironomous larvae, on commercial sclaes for use by ordinary farmers engaged in farming.

Microworm culture-Panagrellus redivivus

Microworms which are white in colour and grow upto 3mm in length, are excellent live feed for the fish fry and are very easy to raise. Microworms are live bearing, releasing 10 to 40 young every 1 to 1.5 days for a 20 to 25 day life span. Therefore, each female produces approximately 300 young. The young reach sexual maturity in approximately three days. Their size increases by three times during the first day and five to six times during the next three days. The live nematodes contain 76% water and 24% dry matter; 40% of the dry matter is protein and 20% is fat. Trial culture of bread worms were done by employing different methods. Microworms are especially useful for species of fish whose fry are too small to initially to feed on newly hatched brine shrimp nauplii. They can be used as an alternative or substitute to baby brine shrimp.

Fig Panagrellus redivivus cultured on bread/ milk substratum

Grindal worm

Grindal worm (*Enchytraeus buchholzi*) is a small white non-parasitic worm that lives in soils around the world. They are similar to White worms that are commonly used by other aquarists as a source of food for their fish. However, these worms are smaller than White worms and their generation and maintenance differs slightly. Grindal worms are good food source for young fry and adult fish, that stay under a couple of inches.

Infusoria

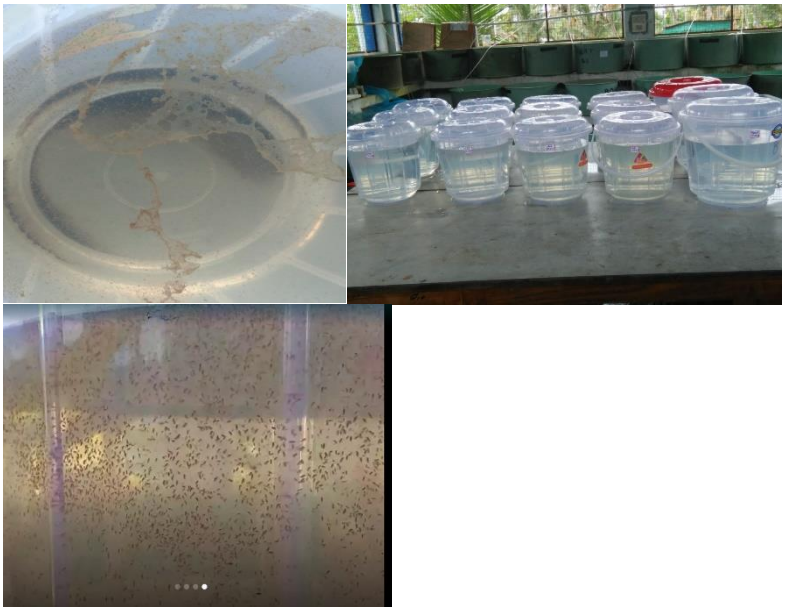
The production of infusoria on different substrates namely Banana peel
Potato peel, Cabbage leaves, Tomato slices and lab beanswere assessed and it was seen that infusoria production was high in cabbage and the next highest production was in banana peel

Paramecium

Paramesium cultivation was carried out on banana peels, milk and potato peels. Faster growth of Paramecium was recorded in milk enrichedmedia. The study showed easy maintanence of paramesium and alos highlighted the nutritional value of Paramecium was good

Zooplankton

Zooplanktons collected from freshwater ponds using plankton net. Zooplankton such as cladocerans, copepods and rotifers were isolated from the water sample and maintained using Baker's yeast Saccharomyces cerevisiae as feed. Experimental trials were designed to study the effect of different plant sources (coconut oil cake, GNOC, Sesame oil cake and rice bran) on the population density of zooplankton.Moina showed adequate growth on both CSOC and GNOC and advanced growth and coloration exhibited by Moina fed with GNOC.



Experimental culture of *Moina micrura*

Enchytraeus buchholzi cultured using wet sponge as substratum and oats as food

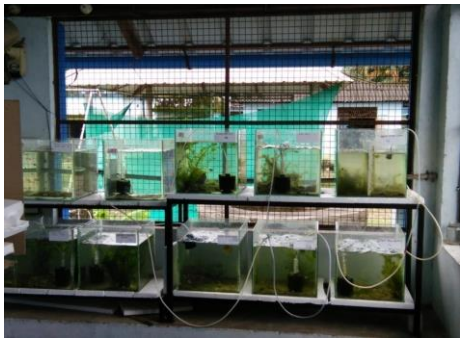
Blood worm / Chironomous larvae

Chironomous larvae are most abundant aquatic insects found in any water body and it is common for chironomids to comprise more than 50% of species, some kinds are blood red due to the presence of haemoglobin.The chironomid larvae and pupae are highly nutritious, easy to rear and their peculiar movement will induce feeding stimuli in fishes.The larvae can be used as live food for aquarium as well as carnivorous fish fry in aquaculture.

5. Establishment of fish seed bank

Timely availability of quality seed is a major factor influencing the success of any aquaculture enterprise. The culture of ornamental fish continues to be an important activity in the country. Attractive coloration determines the commercial value of ornamental fishes. Quality of brood stock and larvae is a crucial factor in ornamental trade. The project was aimed at facilitating timely availability of seed, evaluate and formulate different brood stock and larval diets and to Standardize of different approaches for improving larval growth and survival. Sixteen varieties of 9 types of fishes as given below were studied.

	Fish	**Variety**	**Scientific name**	**Generation under study**
1.	Gyppy	Half black guppy	*Poecilia reticulata*	F7
2.		Seven color guppy	*Poecilia reticulata*	F5
3.		Yellow cobra guppy	*Poecilia reticulata*	F7
4.		Turquoise red tail guppy	*Poecilia reticulata*	F7
5.		White tuxedo guppy	*Poecilia reticulata*	F5
6.		Yellow tuxedo guppy	*Poecilia reticulata*	F7
7.	Molly	Pepper molly	*Poecilia velifera*	F2
8.		Black molly	*Poecilia sphenops*	F2
9.	Tiger Barb	Tiger barb	*Puntius tetrazona*	F1
10.	Fighter fish	Siamese fighter fish	*Betta splendens*	F2
11.	Sword tail	Sword tail	*Xiphophorus hellerii*	F2
12.	Platy	Red coral platy	*Xiphophorus maculatus*	F3
13.		Red top platy	*Xiphophorus maculatus*	F3
14.	Gold fish		*Carassius auratus*	F1
15.	Tilapia		*Oreochromis niloticus*	-
16.	Asian cat fish		*Pangassianodon hypophthalamus*	-



Comparative evaluation of the color enhancement potential of different pigment sources of plant origin in the gold fish *Carassius auratus*

The experiment was designed to study the development of color in gold fish fed carotenoids from three different plant sources,namely African tulip tree flower(Spathodea companulata),Red bell pepper(Capsicum annum) and Pomgrenate peel(Punica granatum).The pigment sources are included in feeds, at 5%inclusion level. The water quality was maintained by periodic partial replenishment, as required. Water quality parameters were monitored over the duration of the study by sampling at fortnightlyintervals. The carotenoid content and the colour intensity, was estimated using spectrophotometer. The highest carotenoid value was obtained from red bell pepper.

Difference in color intensity shown by ***Xiphophorusmaculatus***fed withexperimental diets – control,muringa, shrimp head waste meal, mixed diet of moringa, shrimp head waste meal at 1:1

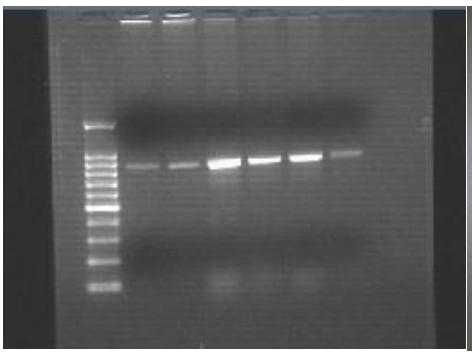
Difference in Color intensity shown by *Carassiusauratus* fed with experimental diets - control feed, African tulip tree flower, pomegranate peel feed and Red bell pepper respectively

6. Establishment of a fish virology/cell culture lab

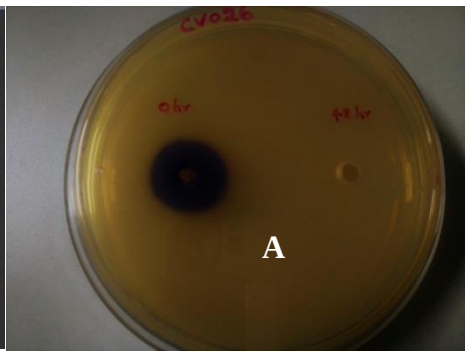
A fish virology/cell culture lab was established in the Centre for Aquatic Animal Health Management. Cell lines from different tissues of Channa diplogramma was developed and primary cultures were developed from caudal fin, spleen, and eye. Only 17.74% of the total explants showed radiation of the cells from the fragment of the tissue. Among this, only 8 explants formed the monolayer leading to the development of cell lines.

7. To screen the probiotic potential of N-acylhomoserine lactone-degrading bacteria isolated from fish culture pond and gastrointestinal tract of *Oreochromis niloticus*

Diseases constitute one of the most important economic constraints for the development of aquaculture sector. With a view to control bacterial diseases in aquaculture, 20 bacteria capable of inhibiting the quorum-sensing signals (Quorum quenching bacteria) were isolated from fish intestine and soil from culture pond. The potential probiotic traits of these bacteria are being investigated to explore their use as a suitable biocontrol agent in aquaculture. The presence of an autoinducer inactivation homologue gene (Fig) and AHL-inactivation assay showed the ability to rapidly degrade synthetic C6-HSL and C10-HSL in vitro and hampered violacein production. They had excellent biodegrading ability of natural N-AHL produced by Aeromonas hydrophila, suggesting that they can be used as a potential quencher bacterium for disrupting the virulence of A.hydrophila. The study indicates that the combination of quorum quenching bacteria could be used as a non-antibiotic feed additive in aquaculture to control bacterial diseases.



PCR detection of aiiA homologue gene. Lane A: 100 bp DNA ladder (Promega); lane B-G: different isolates; lane H: negative control. Arrow shows the expected amplicon size of approximately 800 bp.



AHL degrading activity of QQ isolateon natural N-AHL. QQ isolate was incubated with crude cell free culture supernatant of *A.hydrophila* (served as natural N-AHL) for 0 h (A) and 48h (B). Pigment formation indicates the presence of N-AHL in the cell free culture supernatant of *A.hydrophila*; degradation of N-AHL is evident by loss of pigment formation on the biosensor lawn

8. Molecular characterization of multidrug resistance genes

Antimicrobial resistance is a major global public health concern and considered as a serious food safety issue. Samples were collected from different area and compared with samples from a control area. Samples -water, soil, clam, fish, shrimp and crab - included the direct discharge from the hospital to public water bodies and the aquaculture farms using the same water as inlet water source. A total of 324 isolates were collected from different samples. Most of the isolates belonged to Gram negative Enterobacteriaceae, Pseudomonas, Bacillus, Aeromonas, Acinetobacter, Staphylococcus, Streptococcus and Enterococcus families.

The antibacterial activity of over 30 antibiotics was determined by MIC and antimicrobial susceptibility (AST) was also determined. Results showed high resistance against many antibiotics. Maximum resistance was shown by Staphylococcus and Enterobacteriaceae group. MIC concentration was also found to very high. Most of the Enterobacteriaceae group showed MIC value of above 240 µg ml-1. Screening of antimicrobial resistant gene showed positive result for methicillin (mecA gene), vancomycin (van B, vanC, vanH, vanX), and fluoroquinolones (qnrA, qnrB, qnrS) and extended spectrum beta lactamase resistant genes (blaNDM-1, SHV, CMY, CTX-M, TEM). Vancomycin resistance was successfully transferred from the resistant strain as a donor to a vancomycin sensitive recipient indicating the possible plamis mediated transfer of AMR.

9. Advisory services and technical consultancy to farmers & government

Whenever disease was reported by the farmer; steps were taken to advise the farmer to take prompt treatment measures and to collect samples. Farmers were encouraged to practice BMPs.

10. Diagnostics services for fish diseases

Controlling disease in shrimp culture can benefit the shrimp and fish farmers of Kerala in a significant way as disease is now regarded as the most important constraint to production. Farmers can ensure the stocking of disease free healthy shrimp/fish seed, an essential pre-requisite for health management in aquaculture, by getting the seed tested for its health before stocking. During culture operation, prompt and timely diagnosis can be done at the centre so that the farmer can take immediate preventive steps to control the spread of infection, if any, thereby minimizing significant revenue losses that would normally occur in such circumstances. The samples were screened by PCR for the presence of White spot syndrome virus (WSSV), Monodon baculo virus (MBV), Infectious hypodermal haematopoetic necrosis virus (IHHNV), Hepatopancreatic parvo virus (HPV), Enterocytozoon hepatopenaei (EHP), Tilapia lake virus (TiLV), Epizootic ulcerative syndrome (EUS), Infectious myonecrosis virus (IMNV), Yellow head virus (YHV), Taura syndrome virus (TSV), Macrobrachium rosenbergii nodavirus (MrNV) and Extra smallvirus (XSV).

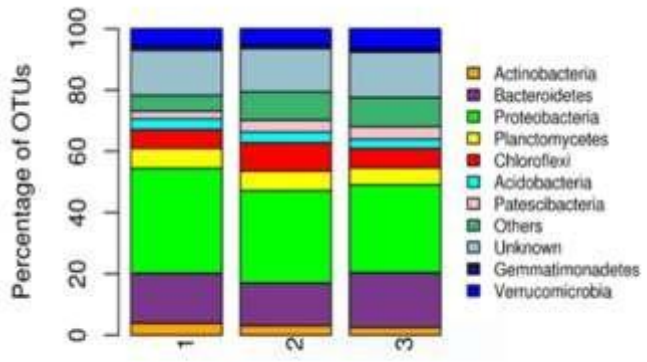
Research progress during 2019-20

Microbialdiversityandabundanceofnitrifiersin aquaponicsystems

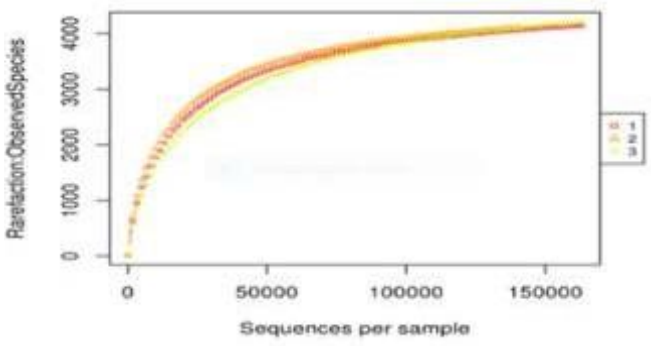
TheDNA was extracted from the filter bed of 3 commercial aquaponic systems and microbial diversitywasanalysedusingmetagenomicanalysis. The functional gene amplification of nitrifiers was done and their abundance using real time PCR. Functiongeneanalysisdemonstratedthepresenceof bacterial ammonia oxidizers (bacterial *amoA* gene), archaeal ammonia oxidizers (archaeal *amoA* gene) and nitrite oxidizers (*norA*) among all the sample. Real time qPCR also indicated high abundance of bacterial and archaeal ammonia oxidizers in aquaponic filter bed.

Metagenomic analysis of microbial diversity

Metagenomic analysis of three sample shows proteobacteria as abundant phyla

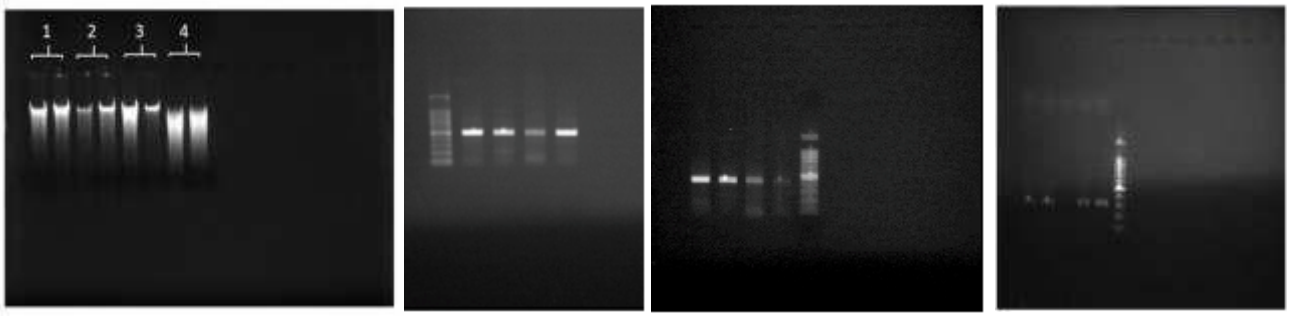


Relative abundance at phylum



Observed Species curve obtained for the

Functional gene analysis of nitrifiers



DNA Extractionofsamples BacterialamoAgeneArchaealamoAgenenorAgene

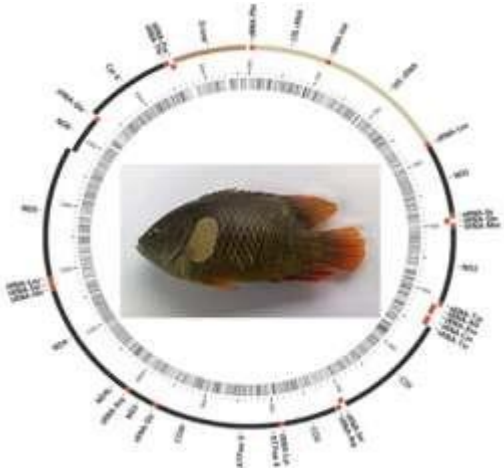
Abundance of nitrifiers in 3 commercial aquaponic systems

Functional Gene	Sample 1	Sample 2 Vytilla	Sample 3

	Amballore		Puthencruz
Bacterial *amoA*	7.3 x 10^2	2,2 x 10^2	4.6 x 10^2
Archaeal *amoA*	8.7 x 10^2	7.3 x 10^2	1,45 x 10^2
Bacterial *norA*	0.62	1,135	1,0

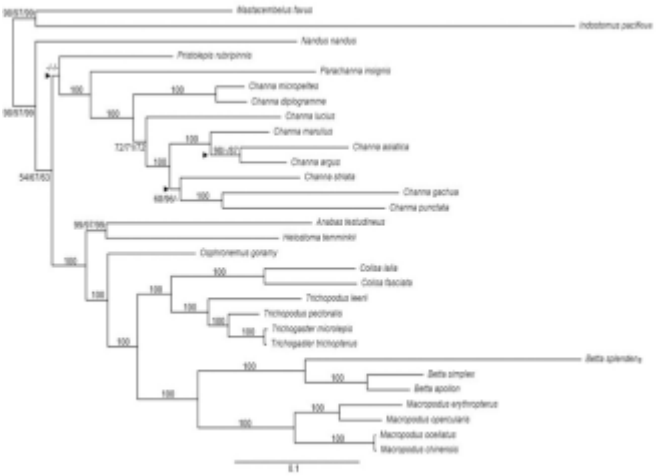
Development of molecular markers for the conservation of fishes endemic to KeralaMitochondrial genome sequencing of *Pristolepisrubripinnis*

Pristolepisrubripinnis, currently known only from the Pamba and Chalakudy rivers in Kerala, India, is distinguishedfromallotherspeciesofthegenusbyits color pattern, which includes orange-red soft dorsal, soft anal and caudal fins, and a yellow to orange pelvic fin. This is the first complete mitochondrial DNA sequence of a species of the family Pristolepididae.Completemitogenomephylogenyof Anabantiformes shows Channidae as sister family to Pristolepididae



Complete mitogenome of*Pristolepisrubripinnis*

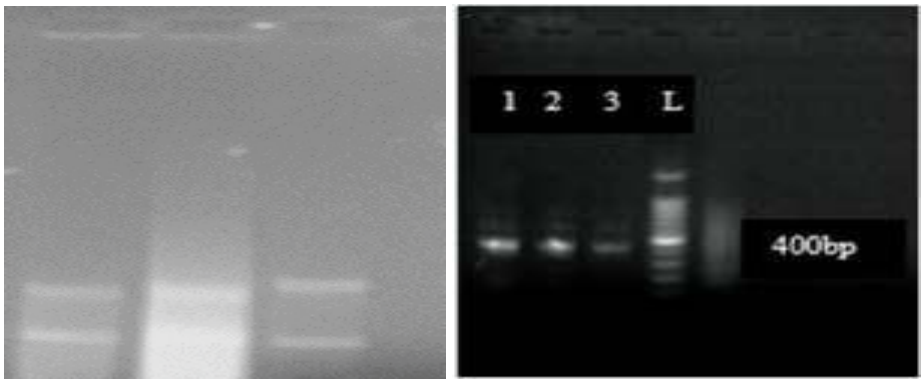
The organization and characterization of the complete mitochondrial genome of *P. rubripinnis*was carried out.

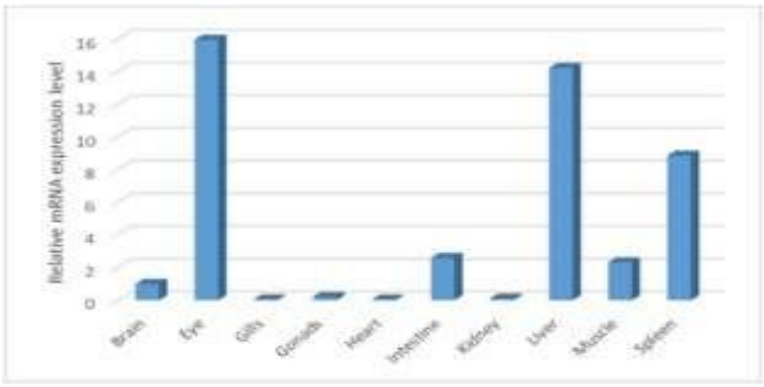


ıylogeny of *Pristolepisrubripinnis* among

Molecularcharacterizationofinsulinlikegrowth factor1(*igf1*)geneinEtroplussuratensis

The expression of genes in the IGF signalling are usedto validate growth, development and reproduction of Etroplussuratensis. The amplification and characterization of igf 1 gene in Etroplussuratensis was analysed and the expression patternsofigf1geneindifferenttissueswasanalysed using the extracted RNA, cDNA construction using reverse transcriptase PCR, Primer designing for Amplification of *igf 1* gene, Polymerases chain reaction, Expression analysis of *igf 1* gene using rapidamplificationofcDNAends.





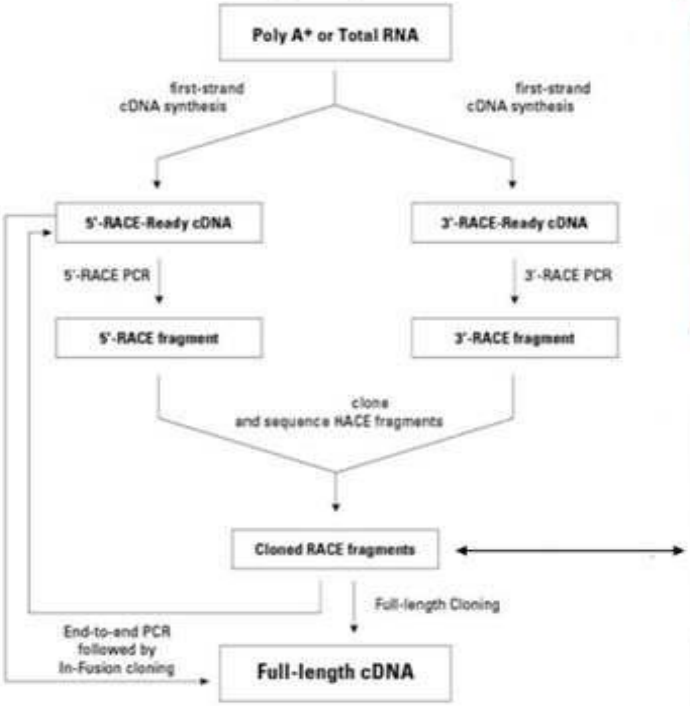
RNA bands in samples Amplification of *igf 1*

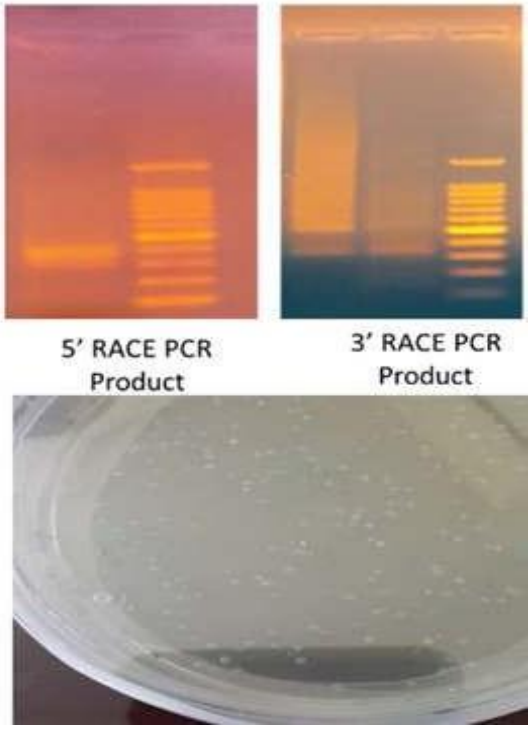
geneRelative expression of *igf 1* gene

The expression of igf1 gene was further confirmed withrealtimePCR.Thehighest-levelexpressionwas detected in liver, eye, spleen, intestine, muscle and braintissue.Lowlevelofexpressionwasseeningills, heartandkidney

Rapid amplification of cDNA ends (RACE)

- To obtain the full length sequence of an IGF 1 transcript found within a cell





Developing brood bank for sustainable production of Karimeen, *Etroplussuratensis* (Bloch, 1790) and an initiation of selective breeding program.

During the third year (2019-20) attention was given to refine the breeding and rearing protocols, water quality management and rearing juveniles in various systems like tank, ponds, RAS, biofloc etc. Brooder tanks were installed with innovative recirculation system with MBBR media and this improved the spawning by avoiding frequent water exchange. A new water circulation system was established in outdoor tank with swirl filter and grow bed to rear aquatic plants. Larval rearing in glass tanks were also done successfully with more than 90% survival with reduced heterogeneous growth.

Complete hatching was achieved using eggs separated from substrata and was a breakthrough. Research is progressing on the use of various smicroalgae and zooplankters in improving larval growth and survival. Studies on the F1 and F2 generation are continuing to find the influence on in breeding on egg quality, larval survival, growth and spawning. Microalgal lab started working and stock culture of various strains arekept.

Rearin /s

A rear b

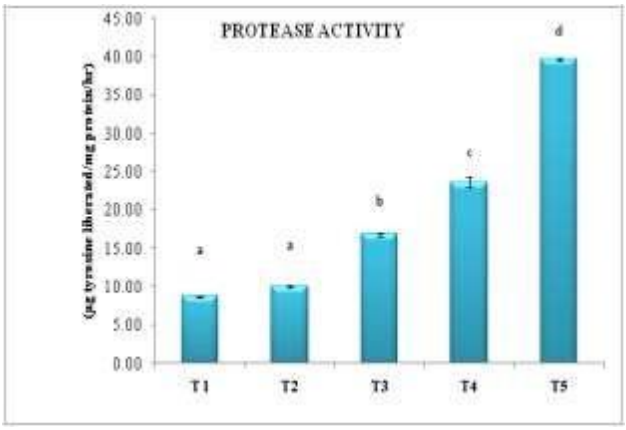
content of 41.48%, crude lipid of 8.10%, Crude fibre of4.06%, nitrogen free extract of 25.98% and ash content of 12.49%. The growth and survival of the crab was analyzed in different weaning system adopted with difference in enclosure and feed type namely treatment I, II, III &IV. The percentage weight gain showed an increasing trend initially and then gradual decrease during the period of culture. The study indicatd the effectiveness of formulated feed using locally available protein rich ingredients and crab holding enclosures on growth and survival rate in juvenilemudcrabculture.

Evaluationofcashewnutprocessingwaste(CPW) asanonconventionalfeedingredientinthedietof tilapia, *Oreochromismossambicus*

Five iso-nitrogenous (30% CP) and iso-lipidic (6%) feedswerepreparedwithgradedlevelofreplacement of soybean meal with cashew nut processing waste (CPW). The feed ingredients were mixed as per the feed formulation and autoclaved at 105^{O}C for 15 min and allowed to cool. Then the vegetable oil, vitamin and mineral mix, salt, yeast and carboxy methyl cellulose (CMC) were added and pelletized using hand pelletizer with 1mm die. The pellets were oven dried at 60^{O}C and kept in the air

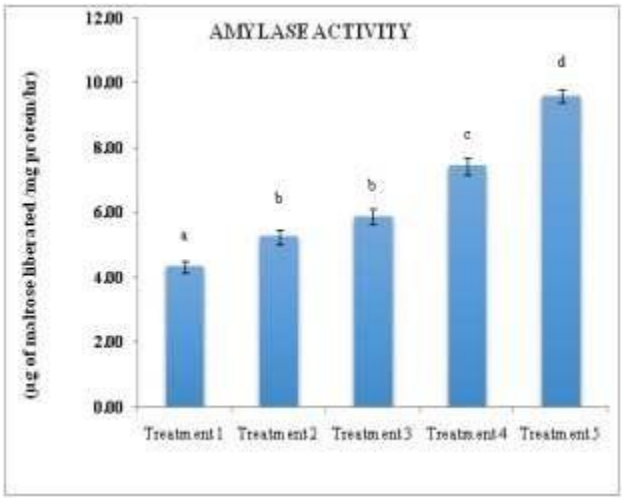
tight container for furtheruse.Thetilapiawasfedfivefeedsintriplicate groupsthreetimesdailyuptosatiationat10,13,and 17hrsfor60days.Fecalmatterwascollecteddailyfor digestibility studies. Growth, nutrient utilization, blood biochemical parameters, digestive enzymes, digestibility and liver and intestine histology were checkedtodeterminetheeffectoffeedontilapia.

The treatment fed with 50% replacement of soybean mealshowedhighestvalueoffinalweight(70.83g)at the end of the experimental period. The maximum SGR (1.96) was recorded with 50% replacement of soybean meal which was significantly higher (P<0.05), significantly lower (P<0.05) FCR, significantly higher (P<0.05) HIS and significantly higher (P<0.05) CF among the treatments. Viscero somaticindex(VSI)andsurvivability



did not change (P>0.05) among the treatments. CPW could be used as potential fish feed ingredient. It can be incorporated up to 40% along with other plant mixture in tilapia diet for better growth and nutrient utilization.

Protease activity in *Oreochromis mossambicus*(Tilapia) fed graded level replacement of soybean with CPW over a period of 60 days

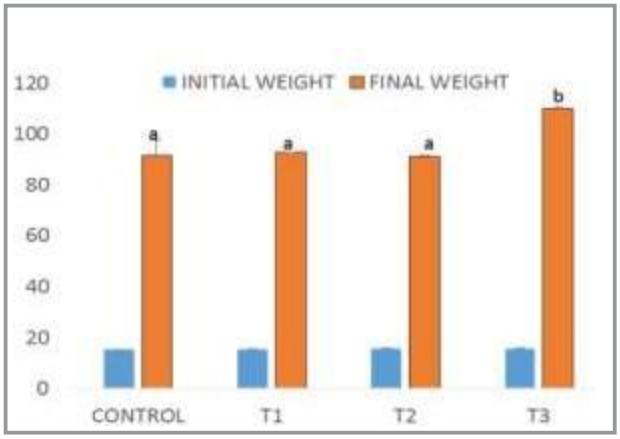


Amylase activity in *Oreochromis mossambicus* (Tilapia) fed graded level replacement of soybean with CPW over a period of 60 days.

Effect of *Cassia roxburghii* as a Phytogenic Feed Additive on Growth Performance, Nutrient utilization and Health status of *Oreochromis mossambicus*

Three experimental diets, T-1, T-2 and T-3 were prepared incorporating 1% leaf, seed and bark extracts(1gofdriedwaterextractpowder)of*Cassia roxburghii*andacontrolfeedwithoutanyextract.20 *Oreochromismossambicus*fry (15.50 ± 0.12 g) were stocked in each tank of 200 l capacity. Triplicate groups of fish were fed till satiation, thrice daily, at 10:00and13.00and17:00hfor60days.Proximate analysesofallfeeds,initialandfinalfishtissuewere determined based on methods described in AOAC (1998). Initial weight, final weight, weight gain, weight gain% and survivability of *Oreochromis mossambicus*were evaluated. FCR, PER and PRE were estimated. Total serum protein, albumin, triglyceride, HDL, VLDL, SGOT, SGPT, alkaline phosphatase wereanalysed using commercial kit (Aura diagnostic). Histopathology of intestine was conductedusingstandardprotocols.

At all the treatments tilapia showed an increasing growth trend over the culture period. The result showed significant difference ($P<0.05$) among the treatments.Thegroupfedwithbarkextractexhibited significantly highest final weight (109.93g). Highest value of weight gain (94.2g) was exhibited by the group fed with bark extract. There was significant difference ($P<0.05$) among the treatments. The value of weight gain ranged between 75.2 – 94.2 g. The result showed significant difference ($P<0.05$) among the treatments with highest weight gain% (598.71%) in the bark extract fed group. The other three groups exhibited similar but lower weight gain percentage.Fish group fed with *Cassiaroxburghii* bark extract exhibited maximum final weight,weight gain, weight gain percentage. The nutrientutilization factors like PER, PRE also exhibited significant increaseinthebarkextractfedgroup.



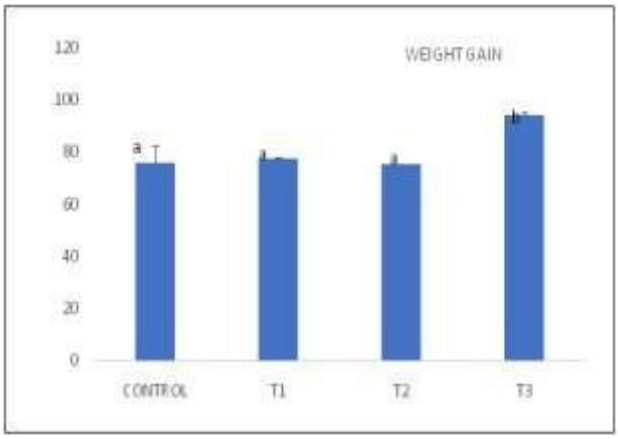


Figure showing initial weight & final weightFigureshowingweightgain

Dietary taurine requirement *of Pangasidon hypophthalmus* fry, based on growth, nutrient utilization, digestive and antioxidant enzyme activities

Dietarytaurine(tau)requirementof *Pangasidonhypophthalmus* fry (0.8g±0.6) was quantified by feeding six isonitrogenous (370 g kg^{-1} crude protein) and isolipidic (10g kg^{-1} crude lipid) casein based purified diets with graded levels of tau. Fish were randomly stocked in triplicate groups in 100 L plastic tubs and fed to apparent satiation over twofeedingsat10.00and16.00hrsdailyfor45days. At the end of the experiment, growth, nutrient utilization, body composition, digestive enzymes, antioxidant enzymes and hematological parameters werestudiedinordertocheckttheeffectoftaurine.

Significantly ($P<0.05$) lower growth and low feed utilization were observed in pangasius fed with no taurinediet.Thefinalweight,weightgainandweight gain%increaseinresponsetodietarytaurineupto15 g kg^{-1}. The feed conversion ratio (FCR) and Protein efficiency ratio (PER) of the fish in different treatmentsrangedfrom1.01to1.57and0.21to0.46, respectively. Results of the current investigation revealed the essentiality of the dietary taurine supplementation for maximizing growth, body composition, digestive enzyme activity and antioxidantactivitiesofpangasiusfry.Onthebasisof results obtained in this study, dietary taurine requirement in the range of 15.03-16.31 g kg-1 is recommended for*Pangasidonhypopthalmus*.

Integrated System for Aquatic Animal Health Research and Management - Establishment of a fish virology lab

A new disease in Anabas was reported. In order to investigatethecauseofthedisease,themuscletissue

explantofthediseasefishwasseeded.Duetotheoily and puss like nature of the explant the whole media turned turbid with bacterial overgrowth with unpleasant viral isolation could not be done. However, bacteriological analysis was carried out andtheisolateswerecharacterized

Establishing best probiotic combination of quorumquenchingbacteriausingRSMmethod

Twenty-two different probiotic feed combinations with four QQ bacteria (*E.faecium, L.casei, L.plantarum & B.thuringiensis*) were designed by using RSM software. Probiotic feed combinations with different cell densities were prepared by inoculating respective QQ isolates in suitable growth medium and incubated at 300C for 48 h. Bacterial cells were harvested at frequent time intervals by centrifugation at 5000 rpm for 5min at 40C. After centrifugation, bacteria were washed twice with PBS and resuspended in PBSmaintainingthe cell densities.Twenty-two combinations of multiple strain probiotic feeds were prepared by adding these cell suspensions at the rate of 1 mLof culture /g of feed to incorporate 104cells/gfeed,106cells/gfeed,108cells/gfeed& 1010 cells/g feed. A binder (Aqua one, Salem Microbes Private limited, India) was used at 1mL/10g feed. Binder alone was added in control feed. After proper mixing of the ingredients, the feeds were air dried. For each quorum quenching probiotic feed, twenty-two groups of 10 goldfish each,*C.auratus*wereintroducedintoglasstanksof 50 L capacity. They were fed with each probiotic diet. Controls were maintained for each of twenty- two probiotic combinations. Feeding was donetwo times daily at the rate of 3% of the body weight of *C.auratus* for 30 days. Continuous aeration and water flow were maintained in all glass

tanks. The activity and behaviour of the fish were monitored andrecordedondailybasis.

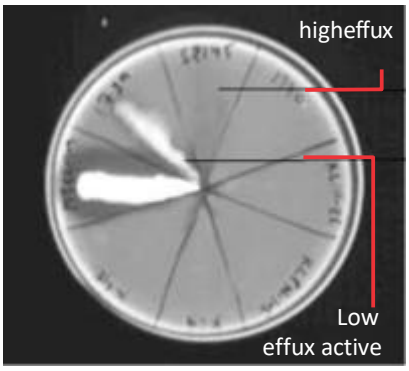
Experimental setup for probiotic feed experiment

Experimental setup of probiotic feed trial

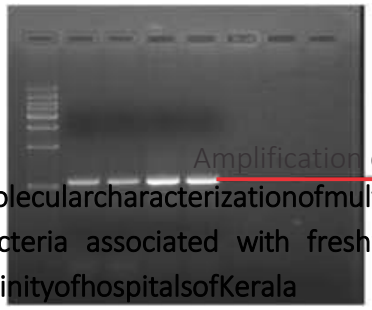
All clinically healthy fish were challenged with *A. hydrophila*.Controls and probiotic fed fish were challenged (10nos/group) via intraperitoneal injection with 0.1mL of 1x 106 cells (LD50 based on preliminary work). The fish wereobserved to determine mortality,externalsignsofinfectionand behavioural abnormalities for two weeks. Dead

fish were removed immediately for bacteriological examination. Bacterial isolation was carried out from hemorrhagic and ulcerative lesions, and from dead fish's visceral organs. All the experiments wereperformed

In triplicate and the data are being analysed



Phenotypic efflux pump detection by EtBr Agar Cartwheel Method



Amplification of OqxAB gene

Molecularcharacterizationofmultidrugresistancegenesand mechanism of transfer among bacteria associated with freshwater and brackish water aquaculture systems in the vicinityofhospitalsofKerala

Antimicrobial resistance is a major global public health concern and considered as a serious food safety issue. Samples were collected from Thalasseri, Ernakulam, Kollam, Alappuzha (closer to hospitals) and Idukki. Samples include the direct discharge from the hospital to public water bodies and the aquaculture farms using the same water for aquaculture. The study indicated that quinolone and carbapenem resistance in EnterobacteriaceaewasfoundtobeveryhighinKollamdistrict. Different types of plasmid mediated quinolone resistance genes such as qnrB, qnrS, qnrA (Fig. 4) and aminoglycoside encoding geneaac(6')-Ib-crwereidentified.Theimportantfindingsofthe studyincludes

(I) (i) detection of the gene OqxAB in 6 E. coli isolates from hospitaldischargepoint(Fig.2andFig.3).OqxABisaplasmid- encoded efflux pump family Resistance-Nodulation-Cell division(RND).TheoverexpressionofOqxABconfersresistant to olaquindox, trimethoprim, chloramphenicol and decreases bacterial susceptibility to fluoroquinolones, thereby increasing the MICs with these agents. In addition, the OqxAB multidrug effluxpumphasalsoledtoareducedsusceptibilitytodetergents and disinfectants, including benzalkonium chloride, triclosane, especiallySDS.

(ii)detectionoftheqacE?1geneinan*E.coli*isolatefromKollam dischargepoint.TheqacE?1geneisatypicalelementofthe3-CS region of the class 1 integron and provides resistance to quaternary ammonium compounds (QAC) located on a mobile geneticelementandcodesforanefflux-pumpmodeofresistance mechanism. The qac genes were most commonly found in Enterobacteriaceae in combination with genes coding for aminoglycoside resistance, chloramphenicol, sulphonamides, trimethoprimandß-lactums.

In order to investigate the reason for the high resistance showed by the isolates from Kollam district to quinolone class of antibiotics, soil samples were collected for antibiotic residue by LC-MS analysis. Among the studied antibiotics, ciprofloxacin residue was detected at the highest concentrations in the soil sample. CIP level was detected in soil sample was 15ppb. Continuouslong-termexposuretothisconcentrationissufficient for the bacteria to develop resistance and is a serious matter of concern, as exposure to concentrations below thesubtherapeutic level over long periods of time may be an optimal condition for selectionandconsequentresistancespreading.

Establishment of Fish and Prawn Seed Bank

The culture of ornamental fish is one of the most economicandprofitableareaoffishfarmingactivities. Poecilid species demonstrate viviparous reproductive strategy with the female storing the transferred sperms within the ovary, followed by internal fertilisation of the eggs.Theprincipalobjectiveofthisstudywastoevaluatethe effectoflivefeedongrowthandreproductiveperformanceof fish.

TheInfluenceofLivefeedSupplementationonGrowth andReproductive Performance of Swordtail (XiphophorushelleriHeckel1848)broodstock

The study was taken up to demonstrate the efficacy of live feed Daphnia for growth efficiency enhancement potentialinswordtailbrooderfish.Theresultsshowed that fish fed with Daphnia two times daily had a mean finalweightof5.97?g,feedconversionratioof1.33and meanfecundityof190embryosperfemale,whichwas significantly higher than the yield from the other two treatments. Feeding with live feed twice a day was foundtobethebestamongthethreetreatments.

word tail-brooder fish Experimental tank set up

Evaluation of growth performance and breeding habits of fighting fish (Betta splendens) under 3 diets and shelters

Various factors affecting the growth and survival of fighting fish larvae, including nutrition, substrate, water quality and stress were studied in separate laboratory aquaria. Three substrates namely thermocol, polythene and banana leaf were tried in triplicate. The effect of three different live feeds namely white worm, moina and bread worm were experimented in a thirty- day feeding trial controlling water quality as pH (7-7.5), temperature (26- 28^{0}C), and dissolved oxygen (5mg/L). A strong positive relation was observed between the temperature and bubble nest area. The best temperature for bubble nest construction was noted to be 28^{0}C. The results indicated that thermocol to be a better substrate considering bubble nest area and fry survival (80-84%). Among the live feeds white worms gave good results followed by bread worms and Moina.

Fighter fish

Fighter fish breeding

Bubble nest formation

Moina culture

Young ones

Bread worm culture

White worm culture

Effect of frozen white worm Enchytraeus sp.) on growthofplaty(XiphophorusmaculatusGünther, 1866)

Astudywasconductedtoevaluatetheperformance offrozenwhitewormsfornutritiontakingplatyfry in laboratory aquaria. The three

experimental diets considered for the study include (frozenwhiteworm (Treatment T1), 50% frozen white worm combined with50%commercial fishfeed (TreatmentT2)andcommercialfishfeed(Treatment T3).ThecultureoftheEnchytraeussp.wasfedonce a day with oats suspension. The results of the study indicatedthatfeedingwithacombinationofwhiteworms along commercial feed Treatment T2 (50% white worm: 50% commercial feed) gave superior response.Thistreatmentalsohadeconomicbenefit.

White worm culture

Experimental play fish

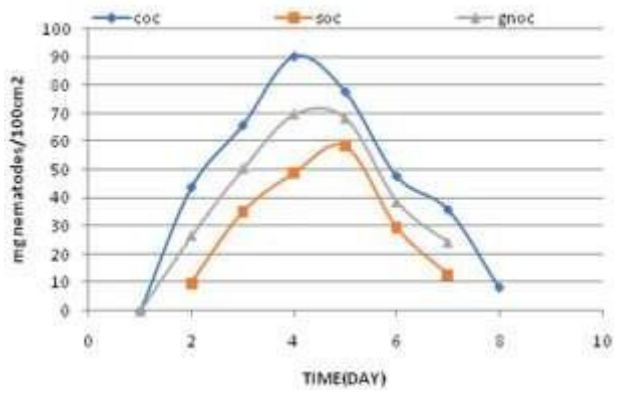
Development and standardization of farmer friendlycost-effectivetechniquesfortheproduction oflivefeedsforaquaculture

The project was started with the objective of optimizing culture requirements for different live feedsandfacilitatetheirmassproduction.Livefeeds are essential for larval rearing of many cultured speciesandcannotsurviveoninertfeeds.

Sour paste nematodes (*Panagrellus redivivus)* are free living, microscopic white worms. Their small size and active movement make them an ideal food for first feeding larvae. Sour paste nematodes are tolerant to a considerable range of temperature and pH. Growth response of *P. redivivus* was assessed employingthreedifferentsubstrates,namely,coconut oilcake (COC), groundnut oilcake

(GNOC) and sesame oilcake (SOC). Trials were doneintransparent rectangular plastic containers. Oilcake(s) soaked and made into a paste, spread evenly over the bottomofthecontainerwasusedasthesubstrateat4-6 cm thickness.Pure culture of P. redivivus was used as the inoculums. Each trial was done in triplicate.Milk was added to the substrate on a daily basis. Biomass production was in the order COC > GNOC >SOC. Maximumproductionwasonday10inCOC,day8in GNOCandonday13inSOCtreatment.Themaximum nematode yield over the harvest period for the COC treatment (97.7mg/100 cm2) followed by GNOC (90.7

mg/100cm2)andSOC(65.13mg/100cm2).

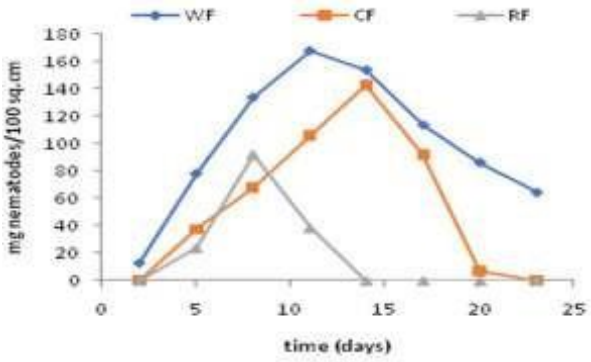


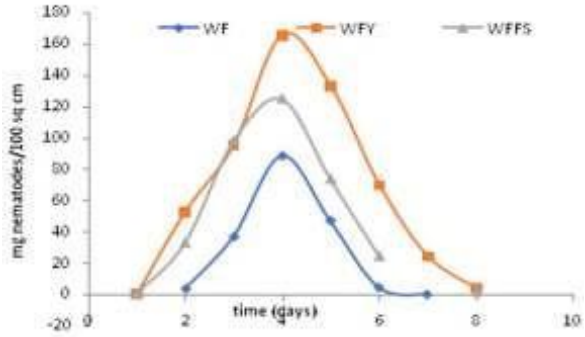
Yield of nematodes (mg/100cm2) cultureddifferent media

Biomass production of nematode on different media

Nematodes are an extremely diverse of organisms, occupying a vast number of ecological niches. They are found in all freshwater, marine and terrestrial ecosystems. Three substrates were assessed with respecttotheirpotentialinbiomassgenerationofsour pastenematodeviz.,wheatflour(WF),riceflour(RF)andcornflour(CF)intransparentplasticcont ainersin triplicate.Pasteofthemediawasspreadonthebottom ofthecontainer.Pureculturereservedastheinoculum. Suspension of the respective medium was added to theculturecontainersoncedaily.Biomassproduction was in the order WF > CF >RF. The maximum production was on day 12, 15 and 18 in WF, CF and RF treatments respectively. All investigated media were found to be suitable for nematode production,

but the wheat flour medium was most effective in termsofnematodeyield.





Potentialofwheatflourasasubstrateforproduction of*P.redivivus*,wasassessed.Wheatflourwastestedin three forms, wheat flour alone (WF), wheat flour in combination with yeast (WFY)and wheat flour in combination with fish organic silage (WFFS). Maximum daily production of nematodes was in the order WFY>WFFS>WF. Harvest of nematodes continueduptoday23inWFY,whileitwas19daysin WFFSand15daysinWFtreatmentsrespectively

Infusoria culture using organic substrates

Infusoria was cultivated in four differentcombinations of substrates namely, Indian Almond leaves,Bananaleave,StrawandCombination(Indian almond leaves + banana leaves + straw). The results indicated that infusoria production was high in

combinationtreatmentfollowedbystraw

ProductionandnutritionalstatusofGrindalworms Enchytraeusbuchholzifeddifferentlow-costfeeds

Grindal worms (*Enchytraeusbuchholzi*) are a small whitenon-parasiticwormthatlivesinsoilsaroundthe world. The nutritionally they are rich in protein withaverage composition as 80.2% water, 58.58% protein, 27.7% fat, 8.58%ash on dry matter basis. Grindalwormsarebeingculturedinmoistsoilbyusing sugar cane bagasse, beaten rice, vegetable waste and feedpelletsasfeedsource.

Culture of vinegar eel in fermented acidic medium

VinegarEels(*Turbatrixaceti*/Vinegarnematode)are infactanematodeandarefree-living,harmless,non- parasitic nematodes which feed on the microscopic culturewithinthevinegar.Thevinegareelsareideal foodforfryastheyarenolargerthan2mminlength and are quite easy to cultivate at home. The vinegar eel was cultivated in 5 different substrates namely Apple+yeast+sugar+water,Lemon+yeast+sugar + water, Grapes + yeast + sugar + water, Orange + yeast + sugar + water and Pomegranate + yeast + sugar + water maintaining the pH of the medium in acidic pH ranges from 4.0 - 5.5.

The growth of vinegar eel could be seen only in the substrate Apple +yeast + sugar + water. Themedium could be little expensive and alternated economic mediaarebeingworkedout.

Utility of chironomous larvae as live feed in aquaculture

Bloodworms can refer to a few different types ofwormincludingthetwomostpopularones;thesmall red larvae from midge flies (Chironomidae) and the genusGlyceraagroupofwormsthatareusuallyfound inmarinewaters.Theworkwasstartedtoexperiment chironomouslarvaeaslivefeedinaquacultureandto studythegrowthandlifecycleoflarvae.

The bloodworms were raised by employing an indigenous method. Yeast in combination with jaggerywithaddedvitamin-mineralmixwasaddedto generate algal culture. Decayed Eichhornia foliage were used to culture the larvae. Water quality parameters, growth and life cycle of the larvae were studied. The result of the study indicates that Chironomuslarvaecanbeusedasapotentiallivefeed in aquaculture. It can be made available to the fishin threevarietiessuchaslivebloodworms,frozenblood worm and freeze-dried blood

worms. Large scale protocolisbeingdeveloped.

Other related activities

Offering advisory services andtechnical consultancy to farmers & government

Technical advice is being provided to the farmers regularly, both over phone and in person. This has been helping the farmers immensely

Offering diagnostics services for fish diseases, facilities for water quality to farmers

Controllingdiseaseinshrimpcultureisbenefitedthe shrimp farmers of Kerala in a significant way as disease is now regarded as the most important constraint to production. The Centre forAquaticAnimal Health Management has been offering diagnostic facility to the farmers

Training programme on “FARM MADE FEED” at KUFOS Payyannur Station, Kannur

In the training programme 60 progressive fish farmersparticipatedandbookonfarmmadefeed for the culture of carp, tilapia and pangasius in EnglishandMalayalamlanguagewerereleased.