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AQUACULTURE ASIA

Collection of tubifex worms as a livelihood

Integrated rice-fish farming on the apatani Plateau

Cryptic gut microbiota

Endangered catfish culture

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Genetically responsible aquaculture

It has frequently been observed that, when it comes to genetic improvement, aquaculture lags behind terrestrial livestock industries. Pretty much all land-based livestock have been subject to informal selection for literally thousands of years, and a lot of it has been subject to intensive formal selection programmes for decades. For example, systematic efforts to genetically improve broilers started in the 1940s with massive performance gains achieved in terms of faster growth, increased meat yield and more efficient feed conversion.

And ok, there are some reasons for this. Mainly, that aquaculture does not have such a strong tradition in most parts of the world (there are exceptions). And aquaculture as a science-based industry is only a few decades old. It wasn't that long ago that that farming shrimp, for example, was *new*.

But given the clear and unambiguous experience of livestock industries, why is it that aquaculture has been so slow to get on board? You can count on one hand the number of large-scale genetic improvement programmes for mainstream aquaculture species. There are some artisanal breeder societies in some parts of the world, and some private industry programmes underway as well - emphasis on private – but in terms of the accessibility of genetically improved seed to the general farming population, it's not really happening on any significant scale.

In the beginning there was GIFT tilapia, and before that there was more or less nothing. GIFT provided a useful proof of concept concerning genetic improvement in aquaculture, for which a World Food Prize was awarded in 2005. While the official GIFT is still developed in Malaysia, after the fish was distributed throughout the region many other suppliers of 'GIFT' tilapia sprang up. It is not clear what relation the fish produced by third party hatcheries have to the original variety. But if the fish have been produced independently, interbred with other strains or possibly produced without genetic supervision over a significant period of time, then it seems likely that their performance is going to be different. There's no real way for farmers to know what they're getting. An improved 'Jayanti' variety of rohu is under active development and in commercial production in India, and doubtless the same issues will also surface there, although the government has taken steps to protect and restrict usage of the name of the improved variety.

In February ICAR and NACA held an expert consultation in India to try and address some of these issues. Given that genetic improvement requires a long term, ongoing commitment and significant resources to achieve, how can we manage the genetic diversity of the fish we have, let alone seek to improve them in a resource-constrained environment?

One recommendation from the meeting was to investigate the feasibility of setting up networks of small, registered broodstock holdings. Linked via IT systems, such networks could form a virtual aquaculture gene pool that could, collectively, sustain high genetic diversity and long-term adaptive capacity, while checking inbreeding depression. More details are available in the Newsletter.

Simon Wilkinson

Aquaculture Asia

is an autonomous publication that gives people in developing countries a voice. The views and opinions expressed herein are those of the contributors and do not represent the policies or position of NACA.

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NACA

An intergovernmental organisation that promotes rural development through sustainable aquaculture. NACA seeks to improve rural income, increase food production and foreign exchange earnings and to diversify farm production. The ultimate beneficiaries of NACA activities are farmers and rural communities.

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Collection of tubifex worms from the Adi Ganga canal, West Bengal as means of livelihood

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Sample of tubifex in Sri Alam's home.

Openly-flowing sheets of malodorous black water in Kolkata city are obviously a matter of extreme dislike for people, but such water is the lifeline of two communities: Firstly the fish farmers beyond the eastern fringes of Kolkata who depend upon domestic sewage of the dry weather flow channel as a source of nutrients to sustain plankton production in fish ponds/wetlands; and secondly, the semi-poor city people, who harvest the tubifex worms *(Tubifex tubifex)* as a livelihood, from a stretch of foul and derelict waterway named Adi Ganga. The present communication is similar in nature to a publication[1], which had highlighted on the harvest and sale of live tubifex worms by the poor from the heavily-polluted Saigon River, which supports ornamental fish production in and around Ho Chi Minh City, Vietnam.

The Adi Ganga

Adi Ganga is a course of the Hooghly/Bhagirathi River in Kolkata city. The Hooghly flows by West Bengal and forms part of the mighty Ganga downstream. It is a 15.5km stretch of canal from Hastings to Rajpur in outskirts of Kolkata and appears for much of its length like a flowing sheet of wastewater. On its way, it crosses Rajpur, Garia, Tollygunge, Kalighat, Alipore, Khidirpore and Hastings in succession and finally confluences with Hooghly River at the point Dahi Ghat. Several settlements of mostly poor people and cowsheds are established on both banks of its entire stretch; temporary toilets open directly into water of the Adi Ganga. Sewage-fed water becomes enriched with organic matter, favouring the natural production of tubifex worms on the stretch of quiet muddy land on both banks, left uncovered during the ebb tide and in bottom sediment. The availability of tubifex worms provides a livelihood opportunity to poorer local people; particularly it is collected in the 2,000 m stretch between Hastings and Kalighat crematorium.

Use of tubifex for larvae of cultivable catfishes in West Bengal

Apart from being a favoured food of aquarium fishes, tubifex is also crucial as live food for early stages of hatchery-produced economically-important catfishes. In production

Exposed bank of Adi Ganga.

Mud collection - another view.

Sieving mud and detritus from the Adi Ganga.

Silt is the main source of tubifex, collected in a container.

Continuous water-flow system in tubifex containers.

of fry stages of *Pangasianodon hypophthalmus*, minced tubifex worm is used as feed everyday @ 3kg/1,320 m^2 pond in addition to powdered milk suspension and a liquefied mixture of groundnut oilcake, shrimp feed dust and Agri-Min feed supplement from the first until tenth day after stocking *P. hypophthalmus* spawn in earthen nurseries (Sri Babul Majumdar: personal communication). In hatchery conditions, finely-chopped tubifex is supplied from the seventh day onwards to young *Ompak pabda*, twice a day at about 25% of the body weight of spawn up to 15 days[2]. In addition to zooplankton, chopped tubifex filtered through a nylon net, adequately washed in freshwater and disinfected with didecyl dimethyl NH_4Cl has been fed to larvae of *Clarius batrachus*; the fry stages (15 to 45 days) strongly prefer whole tubifex worm and shows best growth and survivability with tubifex, among other feeds used. Better growth from tubifex may be attributed to higher crude protein content (65%) in comparison to other feeds[3].

Working in dirty waters

I conversed with Md. Dastagir Alam and his nephew Mr Sonu Ahmed, residents of Canal Road, Khidirpore, near Hastings on the eastern bank of the Adi Ganga. They have been involved in this profession, collecting, gathering and selling tubifex worms on a commercial basis, since 2003-2004. Every day Sri Alam works for 3.5-4 hours, normally from 10am until 2pm or during the ebb tide covering a distance of 150-200 m. He walks uneasily through foul muddy conditions and collects palms full of thick soft mud from undisturbed exposed banks of the Adi Ganga by scratching over it with fingers quite a few times in many areas. Sediment material lying at 15-18 cm depth beneath water column is also collected by right palm.

After collecting 12-15kg each time in a fine-meshed nylon net (which takes the shape of a white mesh string bag), the entire material is sieved in water at 0.45-0.60 m depth. Clay particles and muck are cleaned and filtered out and the tubifex worms and detritus are retained inside the net. This semi-solid detritus-type material, weighing 1.0-1.5 kg, is kept in large durable translucent plastic packets. The practice of mud collection and sieving is continued many times at different locations and 60-70 kg of material that is retained is accumulated in plastic containers, Sri Alam explained. It is a tedious process of cleaning several kilograms of muck and working in dirty water in the hope of finding tubifex worms. The worms are abundant in organic-rich waters because of the rich food supply, and because they have a high tolerance for low dissolved oxygen conditions.

Isolating tubifex worms

After collection, the entire wet semi-solid silt material is brought at home and evenly spread in a 1.2 m x 0.9 m chamber (temporarily constructed with bricks) with a plastic sheet laid at the bottom. Tube well water is added to a depth of 12-15 cm. A large squarish floating frame made of mosquito net cloth is placed over the spread mass and finally the entire structure is covered with two sheets of old flex material such that the inner surface of flex does not come in contact with net frame. Within next one hour, in conditions of the complete absence of light and when temperature at the bottom starts to rise, tubifex worms leave the silt/sediment material, start surfacing and reach the surface of the net frame, moving through its mesh from below. Sri Alam collects the mass of tubifex over the net frame, washes it 2-3 times with clean water to remove residual mud and packs it in 500 g glass bottles. Each such bottle of tubifex is sold to aquarium shops for Rs 16-18/-. Tubifex is also transported to the site of

Sri Sonu collecting mud from bank.

Silt material retained in nylon net.

Mincing of tubifex worms.

Preparation of tubifex for mincing.

Sieving of initial material in water.

Silt material containing tubifex.

Sri Alam collecting mud material.

60-70 kg of silt, the result of a day's collection.

buyers in plastic containers; 3 litres of clean water is added to every 6 kg tubifex. Some professional ornamental fish farmers in Amtala region of South 24 Parganas procure tubifex worms of Adi Ganga @ Rs 25/- per tea-cup full of mass (Sri Tapan Mondal: personal communication).

In post-monsoon, winter, pre-summer and summer months, Sri Alam gets 6-8 kg tubifex every day from the silt material collected and earns Rs 200-300/- by supplying it to ornamental fish culturists and aquarium shops in the same evening at places like Behala, Amtala, Bishnupur in South 24 Parganas District; Belepole, Domjur, Howrah CTI near Dasnagar in Howrah district and even to C. batrachus seed producers in North 24 Parganas District, West Bengal. The practice becomes more cumbersome during the monsoon months when Sri Alam has to work for 7-8 hours a day at 1.2 m water level, even at ebb tide. His monthly income ranges between Rs 6,000-8,000/- (US$86-100). If not sold completely on same day, Sri Alam stores his mass of tubifex worms in temporary rectangular storage chambers 1.2 m^2 in area with 20-22 cm water depth. In the absence of a continuous mild clean water flow system, he makes water replenishment once in every 45-50 minutes.

Risk and threat involved

According to Sri Alam, this practice begun in 2003-2004 when there was a good demand of tubifex amongst seed producers of *Clarius gariepinus* at some scattered areas of Bongaon, Basirhat in North 24 Parganas District. Early fry of *C. gariepinus* in hatchery conditions were fed with 'blood' material prepared after mincing tubifex worms. Presently seed production of *C. gariepinus* is forbidden in West Bengal but sectors/vocations like ornamental fish farming in backyard ponds and indigenous magur *C. batrachus* seed production in cement cisterns have developed and prospered, where tubifex worms have a significant contribution.

The practice of tubifex collection from Adi Ganga has suffered a setback. Initially 30-40 slum inhabitants in the region between Hastings and Alipore (700-750 m) were involved in it but presently it has come down to 5-6 persons. One had earned Rs 500-600/- / day during 2003-2004 by supplying tubifex to aquarium shops but presently its demand as ornamental fish food and market price has reduced. Protein-rich dry granule-type feed brought into market by Chinese ornamental fish food manufacturers are preferred by aquarium shop owners in West Bengal over tubifex worms. Tiny dough balls made of wheat flour heated in frying pan, dry wheat flour in baked/heated form or pieces of half-cooked chapati roti (flat round bread comprising wheat flour, salt and water cooked on griddle) are also being fed to red molly and other live bearers and gold fish under culture, Sri Alam stated.

Collection of tubifex worms from the sewage-laden waters of Adi Ganga involves high risk of skin infection to people like Sri Alam and others. Small pieces of broken glass, shaving blades, tin sheets, discarded syringes, stitching needles and fountain pen nibs are components of rubbish dumped on the banks of Adi Ganga. These may inflict painful bruises and wounds on those walking barefoot through the muddy waters. Some people have had to leave this profession on account of such incidents.

Scientists of ICAR-CIFA have developed production systems of cultured tubifex in captivity[2, 4], which will serve as a source of supply in addition to that exploited by skilled persons from natural repositories such as the Adi Ganga.

Tubifex worms in ornamental fish farm.

Tubifex stored in C. batrachus hatchery.

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Some facts for the grow-out culture of an endangered catfish, *Clarias magur*

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A haul of C. magur fingerlings suitable for grow-out culture.

India is well established as a leading aquaculture producing country in Asia, with production weighted towards the Indian major carps. The diversification of species and culture systems is instrumental in providing consumers with a wide choice of fish. Research on the aquaculture of minor carps, medium carps, catfishes and air-breathing fishes has opened the opportunity for larger adaptation and culture of a wide range of fish species by farmers. Some varieties of carp and catfish have low growth potential, but their marketing scope is sufficient to make them economically attractive.

Clarias magur is one such catfish, which has immense demand among consumers. Usually demand is satisfied by a combination of wild caught and cultured fish. However, a drastic reduction in wild populations has led to this species being listed as endangered by the International Union for Conservation of Nature (IUCN). Only a limited level of its production from pond culture is available, due to lack of technical back up. But success in culturing this fish has been registered after overcoming various technical problems involved during its production. The article communicates the aquaculture practice of this catfish in captivity.

Characters in favour of *C. magur* as a farmed species:

- It has high market demand and food value.
- It adapts well to a variety of freshwater conditions and can survive in conditions of low dissolved oxygen, as it has an accessary respiratory organ.
- It readily matures in captivity.

Procurement of seed

It is not easy to get sufficient stocking material from nature to start aquaculture of any species. Hence hatchery production of seed is necessary to get a reliable supply of the desired number and size of stocking material. This catfish can be

raised in earthen ponds or cement cisterns by feeding compound feed containing 30% protein to get a suitable size of 100-150 g for induced breeding operations. Suitable females can be identified by observing the bulging abdomen whereas males can be identified from their pointed genital papila during the spawning season (July-August).

Induction

Females needs to be injected with Ovaprim @ 1-1.5 ml/kg body weight and are ready for stripping after 17 h post-injection. The incubated eggs hatch after 25-26 hours, depending on temperature. The hatchlings do not accept any feed until their yolk sac is fully absorbed on the third day of life. Mixed zooplankton serves as the best food for the larvae and fed to larvae from fourth day of hatching. They need good water and aeration during their rearing. The larvae thus reared are harvested after two weeks period. They are transferred to cement nursery and reared with compound feed for 2-3 months to produce fingerlings. These fingerlings are ready for stocking in the ponds to get marketable fish.

Pre-stocking management

Pond size and environment

Either earthen pond or cement cisterns are suitable for its grow out culture of this catfish. The pond sizes usually vary depending on seed availability. As this fish does not perform well at high density during its culture, it is better to opt for a medium size pond of not less than 0.04 ha. Cement cisterns can also be used to raise marketable fish, but the fish grow less efficiently compared to pond condition. Hence the possibility of lower production as well as longer culture period cannot be avoided during its culture in cement cisterns.

Water quality parameters should be with such as pH 6-8, alkalinity >20 ppm, dissolved oxygen > 5 ppm and ammonia < 0.05 ppm for optimum growth and survival.

Pond preparation

Pond preparation is essential to provide optimal conditions to fish for higher growth, survival and yield. Hence perennial ponds must be weed free and dewatered to ensure a predator free environment. Seasonal or dewatered ponds should be manured like a carp nursery to promote natural food production in the pond system. Even though the culture is feed based, these fish efficiently utilise natural foods to supplement their growth and survival in many occasions.

Size of seed

The size of seed plays an important role in growth and yield during the culture period. The possibility of lower growth and survival cannot be ignored if smaller seed are stocked. Hence it is always advisable to stock seed at about 10 g in culture ponds to avoid early mortality and lower growth.

Red patch found on the body of C. magur during its culture.

Stocking of seed

Fingerlings are usually brought from own farm or outside, while stocking for the production of marketable fish. It is essential to ensure that the seed are healthy and free from stress. It is often found that the seed accumulates stress during transportation. Hence the seeds need to be acclimatised in the pond environment for quite a long period to get rid from stress before their release, as this catfish accumulates stress easily. It is advised to undertake water exchange as well as aeration during transportation. A lot of mortality is usually found if they are released immediately after long transportation. It is always beneficial to undertake transport and stocking of seed during the early hours of the day to reduce stress and curtail mortality.

The production of a pond depends on the growth as well as on the number of fish stocked. The growing period is also another important factor for a fish farmer. As this catfish grows slowly, it is always recommended to stock at a lower density like 40,000-50,000/ha. It is always advisable to adopt monoculture of this fish due to its slow feeding behavior.

Post stocking management

Feeding

Even though the fish utilises the limited natural food from the pond, it is essential to supply compound feed, where fish meal becomes a more essential ingredient. Feed containing 30-32% protein is sufficient enough to provide optimum energy for the somatic growth during the culture period. This fish accepts sinking feed of 1-2 mm size. The feed should be provided in feeding tray to minimise the feed loss as well as to reduce feed wastage during winter months by observing consumption patterns.

Environment management

The culture environment deteriorates due to accumulation of metabolites and unutilised feed material as it is a feed based activity. Mostly water parameters like dissolved oxygen or pH are affected seriously, apart from ammonia accumulation during the culture activity. This is more commonly seen in cement cisterns while undertaking growout activity compared to pond conditions. Hence it is essential for intermittent water exchange to give optimum environment for their growth and to avoid fish loss.

Health management

Incidence of disease is often found during winter season or just during the shifting of winter to summer. Diseases like fin rot, ulcers and red patches near the tail or body are found. These can be controlled by frequent water exchange at the beginning of incidence. It is better to segregate the affected fishes, which will restrict further spread of disease. Usually before the incidence, affected fish show slow swimming and low acceptance to feed. Hence it is inevitable to take care of environmental management to restrict the incidence of disease.

Harvest

Culturing at low stocking density gives better fish size compared to high stocking density even though the yield per hectare is lower. Fish of 100-150 g have immense preference among the consumers. The growth and yield pattern of this fish have been evaluated at different densities, which indicated that up to 40,000-50,000/ha are sufficient to produce marketable fish within a year, with a production range of 1.5-2.0 t/ha. The yield can be enhanced at higher density, but with smaller fish. In this situation, the culture period can be increased to harvest bigger fish. But farmers prefer to increase their profit and reduce risk by reducing the culture period.

The time of stocking may be another management aspect to get better growth or yield in this fish. The required size of fingerlings is only available during just prior to winter season. So the growth of fish is hampered during the cold period and the growth accelerates only when the water temperature reaches 27-28°C. Hence the stocking time of the fish should be adjusted so that they get a longer growing period during warmer conditions to reduce the overall culture period or to get higher growth and yield.

The cryptic domain of gut microbiota in composite culture of Indian major carps

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According to official estimates, in the year 2016-17, India's total aquaculture production was nearly 6.5 million tons (~ 57% of the total fish production of 11.4 million tons), which is a remarkable ten-fold increase from 0.63 million tons in 1985. Over the same period, the contribution of poly-cultured Indian major carps has steadily grown from 0.38 million tonnes in 1985 to 3.53 million tonnes in 2015 (60.5 to 67.4% of total aquaculture production). This statistics is definite proof for the continuing predominance of carp polyculture systems in the Indian aquaculture scenario from its inception in the mid-1960s.

Fundamentally, the concept of growing compatible fish species in the same environment was to facilitate efficient use of all the ecological niches within the pond and augment the total fish production per unit area. Keeping this in mind, conventional composite fish culture in the Indian sub-continent included the three major carps that occupy unique feeding niches in the culture pond, namely *Catla catla* (catla), a surface feeder relying on zooplankton; *Labeo rohita* (rohu), a column feeder relying on periphyton and mostly plant matter; and *Cirrhinus mrigala* (mrigal), a bottom feeder relying on detritus. These agastric fish species belong to the same teleost family (Cyprinidae) and share several common features, but they also have certain distinguishable morphological and anatomical specifications that are suited to ingest and process their respective natural diet. For instance, the mouth position of catla is supra-terminal, rohu is terminal and mrigal is sub-terminal. Likewise, they differ in buccal cavity structure, relative length of their highly coiled intestine and gut microarchitecture. So logically, we hypothesise the presence of a host-specific microbiome which colonises the digestive tract with distinct functional relevance. But at present, scientific information on this biological aspect of Indian major carps is meagre and inadequate. Therefore, in this article, we make a strong case for in-depth investigations on the gut microbiota of polycultured major carps by presenting an overview of the present understanding about fish gut microbiome and observations of our preliminary study.

Gut microbiota and their functional significance

The digestive tract of all vertebrates including fish is known to harbour a complex microbial ecosystem with a large, diverse and dynamic collection of microorganisms. Over the course of life, these gut microbes become an integral component of the host animal with intimate host-microbe associations and key roles in the maintenance of normal gut function, physiology and health of the host. This includes their critical role in the digestion of complex nutrients like non-starch carbohydrates, intestinal nutrient acquisition, proliferation of enterocytes (intestinal cells), production of secondary metabolites (such as vitamins) and defence against pathogens (by stimulating the immune system and outcompeting opportunistic pathogens). Recently, gut microbes were also shown to influence food intake, energy homeostasis and weight gain through gastrointestinal chemosensing and nutrient-responsive signalling. Considering the fact that the collective genetic potential (metagenome) of the gut microbiota is several folds higher than the host genome (e.g., the human body contains 10 times more microbial cells than our body cells, with ~ 150 times more genes than our own genome), we are only beginning to understand their impact on host animal health and performance attributes. Lesser known aspects such as horizontal exchange of genes between co-residing symbionts and gene swapping from bacteria to eukaryotes might enable the host to gain characteristics which could help them to adapt to new environments. Thus, host-microbe and microbe-microbe beneficial associations (i.e., mutualistic symbioses) could underlie some of the major transitions in vertebrate evolution and ecology.

Present knowledge of fish gut microbiota

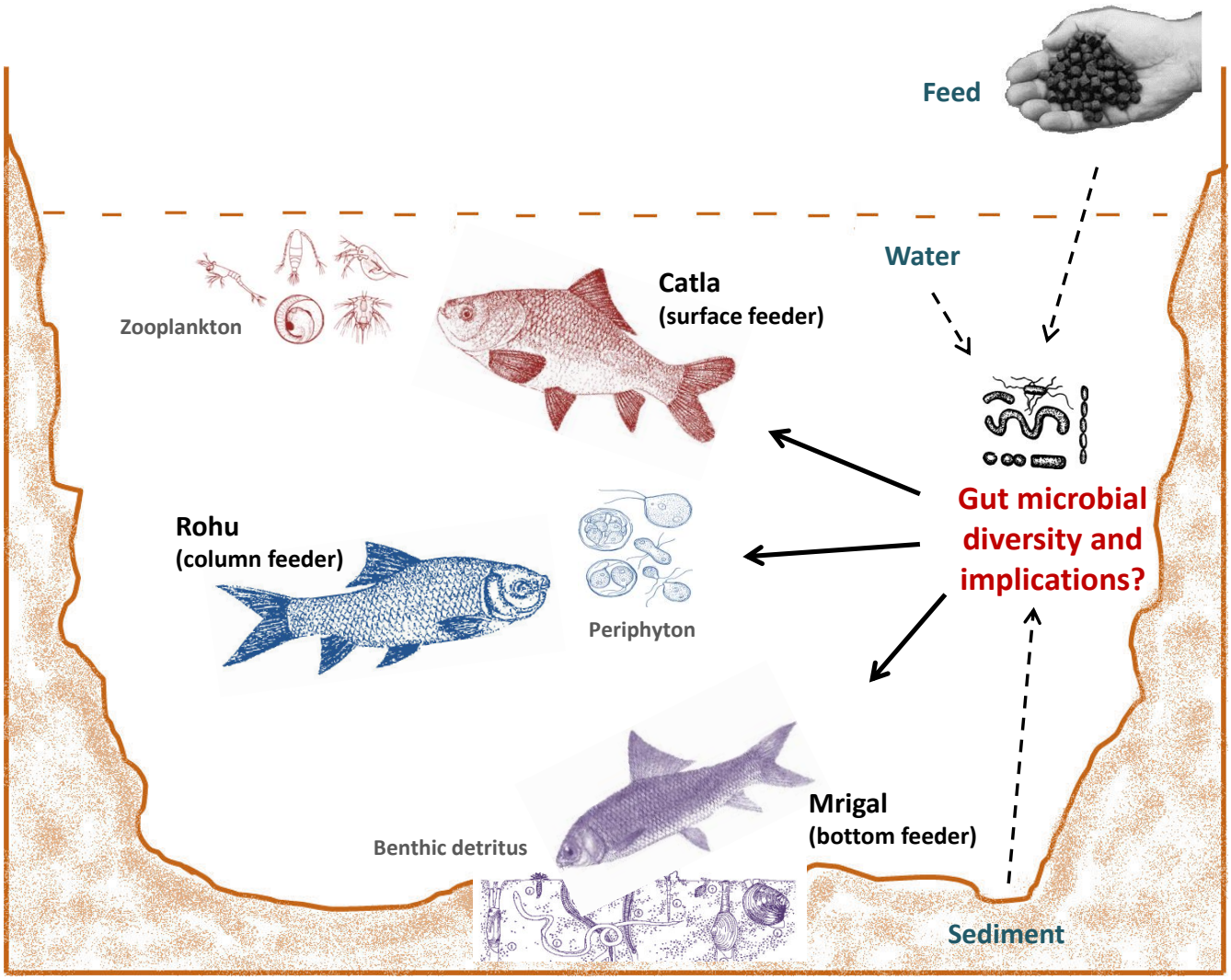
Fish are said to have a simple and less diverse microbial ecosystem as compared to the complex and dynamic one in terrestrial vertebrates. Numerous studies have shown that fish gut is colonised by specialised microbial communities that have not been detected in the environment. Distinct relationships have been found between host diet, trophic level, the anatomy of alimentary tract and gut microbiota composition. For instance, herbivorous and detritivorous fish species are known to harbour distinctive microbial populations due to host-specific selective pressures. Other exogenous and endogenous factors such as developmental stage, age, health status, phylogenetic position (e.g. species-specificity, genotype), domestication process, habitat or rearing environment (e.g. water temperature, salinity, culture system), geographical location, sampling time (i.e., season), starvation and probiotic-prebiotic-antibiotic treatment are also known to affect the composition of fish gut microbial communities. Further, there is evidence for the presence of discrete populations of microorganisms in defined regions of the digestive tract with different metabolic functions and despite high inter-individual variation, a core group of gut microbes has been discovered in some species like zebrafish. So in a nutshell, we know that the gut microbiome of fish is shaped by host genetics, gut physiology, nutritional and environmental factors. Function-wise, in a few herbivorous fishes, gut microbes have been associated with cellulolytic function or high levels of intestinal fermentation to convert indigestible plant matter to metabolically useful short chain fatty acids. Moreover, gut microbial symbionts were shown to be involved in fatty acid and protein uptake in the intestinal epithelium of fish. In terms of phenotype, gut microbiota was found to be associated with growth, anxiety-related behaviour and stress response. With all these findings, our understanding of the genomic, mechanistic and evolutionary aspects of fish gut microbiota is still limited and we are slowly catching up with the large volume of information in terrestrial vertebrates.

Gut microbiome of carps is generally dominated by differing proportions of bacteria belonging to the phylum Proteobacteria, Firmicutes and Fusobacteria, with some species often having a higher abundance of Bacteroidetes and Cyanobacteria. Interestingly, a comparison between growth hormone transgenic and wild type fish indicated that the relative abundance of bacteria of the phylum Firmicutes over those of Bacteroidetes could be one of the factors that contribute to fast growth in growth hormone transgenic common carp. Further down the microbial taxa, the genus *Cetobacterium* and *Aeromonas* are commonly present in the gut microbiota of grass, common, bighead, Asian and crucian carps. Nevertheless, the same rearing environment does not result in similar intestinal microbiota compositions in polycultured carps. For instance, gut bacterial richness was higher in filter-feeding carp than in grazing carp from the same pond ecosystem. Hence, evolutionarily and functionally distinct symbionts could be critical elements in biological differences among carp species. On the other hand, dietary (e.g., nutrient levels and ingredient sources) and environmental factors (i.e., surrounding water and sediment) are also known to be strong determinants of carp gut microbial composition. Further, as in higher vertebrates, the intestinal microbiota of cyprinids have demonstrated significant roles in the digestion of plant material, fermentative metabolism, intestinal nutrient uptake and *de novo* vitamin production.

Technological advances in the investigative approach

Conventionally, culture-based methods and observations were employed to study the gut microbiota of fishes. Bacterial isolate identification based on biochemical and molecular characterisations were time consuming, had restricted discrimination power and also could not provide a complete insight on gut microbial composition and host-microbiota relationships. Further, as the detected species and the number of bacteria was dependent on culture conditions and culture media, our understanding of certain obligate and fastidious anaerobes was seriously limited. In fact, cultivable bacteria represented <10% of the total gut bacteria in fishes, as the majority could not be isolated and cultured under laboratory conditions. In order to overcome these disadvantages and underestimations, several culture-independent molecular techniques have been developed to characterise the microbial community that colonises fish gut by analysing pooled microbial DNA extractions. These methods were relatively

Figure 1: Schematic representation of the context of the study.



bias-free and allowed the identification and determination of the microbial diversity and phylogenetic affiliation of community members, without isolation. Techniques like fluorescence in situ hybridisation (FISH) involves the use of fluorescent-label probes that target specific regions of the ribosomal RNA (rRNA) and facilitates three dimensional observation of specific microbes using fluorescence or confocal microscopy. FISH has been used to track specific probiotics and their intricate spatial relationships with other microbes in the fish gut. For basic analysis of fish gut microbial communities, DNA fingerprinting methods such as restriction fragment length polymorphism (RFLP), denaturing gradient gel electrophoresis (DGGE) and ribosomal intergenic spacer analysis (RISA) have been extensively used. These techniques are based on targeted PCR amplification of variable regions within the ribosomal operons that are unique to bacterial species or strains. They are relatively inexpensive, fairly quick to perform and generally allow medium throughput analysis. The results obtained with these methods provide information on the complexity of the communities but not the specific operational taxonomic units (OTUs) that constitute each community (i.e., they are more qualitative than quantitative). Moving forward, over the past decade, high-throughput next generation DNA sequencing technologies of bacterial 16S rRNA genes have been instrumental in obtaining a comprehensive inventory of gut microbial diversity in several fishes, by identifying large numbers of gut microbial community members at phylogenetic level, irrespective of their biology. The cost-effectiveness and widespread application of this NGS technology and associated computing (bioinformatics) have triggered the generation of huge volumes of information on fish gut microbiome from myriad environments, with a level of detail which was hard to imagine in the past. Further, metagenomic approaches have provided a reference set of several million bacterial genes that allows targeted study of the activity and function of fish gut microbiota. Progressively, meta-transcriptomic discovery of plant biomass degrading capacity from grass carp intestinal microbiomes has also been reported. But surprisingly, to date, no notable culture-independent attempt has been made to elucidate the host-specific gut microbial diversity and dynamics of polycultured Indian major carps.

A glimpse of what is known and what we have observed in Indian major carps

In Indian major carps (catla, rohu and mrigal), hitherto, culture-based identification of few aerobic and facultative anaerobic bacteria has been carried out, either based on biochemical properties or 16S rRNA gene sequence analysis of the enumerated colonies. The microbes that were isolated and genetically identified from Indian major carps belong to the genus *Citrobacter sp., Enterobacter sp.* and *Bacillus sp.* of the phyla Proteobacteria and Firmicutes. Moreover, the hindgut of Indian major carps was found to be highly colonised than the foregut. Some of the isolated microbes were demonstrated to be a distinct source of exogenous digestive enzymes for the host, apparently assisting digestive processes. Apart from this, there is no proper information on 1) the presence of host-specific microbial populations; 2) the source or origin of the gut microflora; 3) the resident (autochthonous) and transient (allochthonous) microbial groups; and 4) the existence of symbiotic host-microbe associations and functional contributions. Therefore, in the following study, we employed both bacterial enumeration and culture-independent DNA fingerprinting (PCR-DGGE) approach to examine the presence of host-specific gut microbiota in IMCs related to their occupancy of distinct ecological niches (figure 1). Also, we looked at the source/origin (from water, sediment and diet) and colonising ability (resident-transient forms) of bacteria in the digestive tract of the IMCs.

Table 1: Details of the sampled fish.

Species	No. of fish	Average body weight (g)	Average body length (cm)	Relative gut length (cm)
Catla	5	76	19.8	5.3
Rohu	4	95	20.8	8.4
Mrigal	5	68	21.8	13.6

Fish used for the study

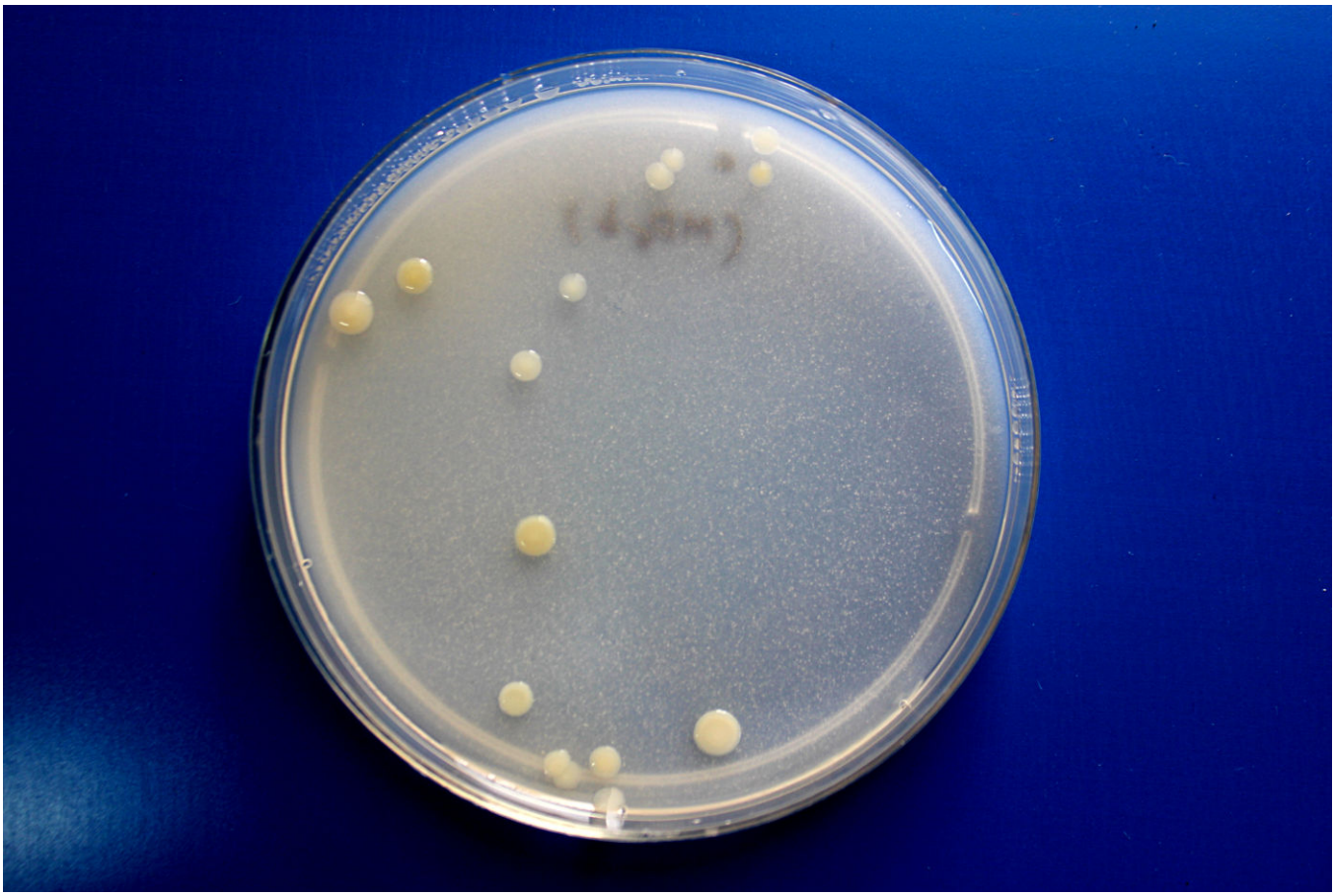
We performed the present investigation on juveniles of the three Indian major carp species, namely catla, rohu and mrigal, which were raised in an earthen carp poly-culture pond of 0.2 ha area and 1 m water depth (Regional Research Station of Central Institute of Freshwater Aquaculture, Bengaluru, India). The pond was annually fertilised with cattle manure and the fish were daily provided a supplementary feed mash made up of rice bran, groundnut oil cake, fish meal and vitamin-mineral mixture. During sampling in the post-summer month of June, the temperature of the rearing water varied between 25 to 30°C. Basic information of the experimental fishes is given in table 1. We observed clear differences in relative gut length between the three species in the following order, mrigal > rohu > catla, reflecting their distinct feeding habits.

Table 2: Cultivable aerobic bacterial count (CFU/g or ml*).

Sample	TPC	Proteolytic	Lipolytic	Amylolytic	Cellulolytic
Water*	7×10^2	7×10^2	5.5×10^2	6×10^2	6×10^2
Sediment	0.9×10^5	1.2×10^5	0.6×10^5	0.7×10^5	0.7×10^5
Feed mash	2.5×10^6	-	-	-	-
Catla digesta	2.8×10^7	2.6×10^7	-	2.1×10^7	2.4×10^7
Rohu digesta	2×10^6	1.5×10^6	0.8×10^6	1.1×10^6	0.3×10^6
Mrigal digesta	2.8×10^6	2.3×10^6	2.4×10^6	5.5×10^6	2.6×10^6
Catla intestine	3×10^6	2.9×10^6	2.5×10^6	2.6×10^6	2.2×10^6
Rohu intestine	4.6×10^5	4.5×10^5	-	6.8×10^5	5.2×10^5
Mrigal intestine	5.6×10^5	6.2×10^5	5.6×10^5	6.8×10^5	5.1×10^5

Figure 2: Collection of fish, intestinal tract and digesta samples.

Figure 3: Methodology for enumeration of aerobic cultivable bacteria.



Sampling procedure

The experimental fishes were randomly collected from the culture pond using drag net, euthanised with cold-shock and transferred to the laboratory. Soon after, they were dissected under aseptic conditions and the entire digestive tract was carefully removed and uncoiled. The intestinal contents were gently squeezed out and collected. Then the empty intestine was washed thoroughly in physiological saline (0.9% NaCl) and pooled together. At the time of field sampling, water and surface sediment samples were also collected from five spatially distinct locations in the same fish pond and pooled

together. Likewise, a small portion of the supplementary fish feed mash was taken for bacterial count and composition analysis. After allocating a small portion of all the samples for serial dilution and bacterial enumeration, the remaining samples were stored at -80°C until DNA extraction.

Culture-dependent approach: Methodology and observations

For enumerating the aerobic heterotrophic bacteria present in the collected samples, first we prepared well homogenised and diluted fractions of each sample (intestine and digesta of the test carp species; water, sediment and feed). From three appropriate serial dilutions (1:10), we took 0.1 ml of sample and aseptically pour plated in sterilised agar plates, in duplicates. We used tryptone soy agar media for total plate count (TPC); and starch, cellulose, peptone gelatin and crude coconut oil agar media to enumerate bacteria that produces amylase, cellulase, protease and lipase, respectively. The inoculated plates were then incubated at 26°C for 24 hours. Subsequently, the plates were examined for the development of bacterial colonies and well separated colonies were counted, multiplied by the correct dilution factor and expressed as colony forming units per g or ml of the sample.

Based on the results of the bacterial enumeration (table 2), firstly, we observe that the bacterial count in the intestine and digesta of all the three Indian major carps was several orders higher than in the water and sediment. This reaffirms the fact that the digestive tract of fish is a nutrient rich abode for microbes than the culture pond habitat. Secondly, the surface filter feeder catla was found to have a higher abundance of gut microbes (both in intestine and digesta) as compared to rohu and mrigal. This corresponds to a previous observation in polycultured Chinese carps, where gut microbial diversity of filter feeding bighead carp was found to be higher than that of grass carp and crucian carp with grazing habits. Between rohu and mrigal, the latter had a relatively higher bacterial count in the digesta samples possibly linked to their detritivorous feeding habit. Overall, this result serves as an indication for host-specific and feeding habit specific selective pressures on the gut microbiota of IMCs, just as we had hypothesised. Thirdly, in all the carps, the bacterial count of intestine samples was less than the digesta samples, implying the possible existence of resident and transient bacterial groups with differences in colonising ability. Fourthly, in all the samples, the order of bacterial cell count was similar between the plates with different nutrient substrates, i.e., amino acids, fatty acids, starch and cellulose. This suggests that the enumerated bacterial strains could use different nutrients for their survival and growth, and perhaps benefit the host by contributing exogenous digestive enzymes.

For microbial DNA extraction, we followed a simple conventional protocol which included mechanical, chemical and enzymatic lysis of the microbial cells. Briefly, 400 µl sample of centrifuged and concentrated organic matter from pond water, sediment and feed; and manually crushed and homogenised digesta and intestine were added to small (2 ml) screw cap tubes containing both 0.1 and 0.5 mm sterile zirconia/silica beads. To this suspension, two volumes of lysis buffer (2% cetyl trimethyl ammonium bromide, CTAB) was added and the mixture was bead beaten twice for 2 minutes in a mini bead beater for mechanical cell disruption. The sample-buffer mixture was then incubated at 70°C for 30 minutes, with occasional mixing for chemical lysis. After that, lysozyme (10 mg/ml) was added and the mixture was incubated at 42°C for an hour for enzymatic cell lysis. After mechanical (beat beating), chemical (CTAB) and enzymatic (lysozyme) disruption of microbial cells to improve extraction yield and the quality of the community DNA, proteinase K (2 mg/ml) and RNase (1 mg/ml) was added to the mixture and incubated at 37°C for 45 minutes, for degrading protein and RNA contaminants. Thereafter, we followed the conventional phenol-chloroform method and the extracted DNA was precipitated with ice-cold ethanol. For each sample, DNA was extracted in four technical replicates, pooled together and purified by gel elution. The freshness of the sample and extraction method was found to be highly critical to obtain good quality microbial DNA. Subsequently, we amplified the bacterial 16S ribosomal RNA gene and V3-V4 conserved regions from each sample using ~100 ng DNA template, universal bacterial primers and Taq polymerase enzymes in a touch-down PCR reaction.

Finally, we employed DNA fingerprinting method (denaturing gradient gel electrophoresis of the amplified V3 region of the 16S rRNA bacterial gene) to generate preliminary information on the bacterial diversity of Indian major carp intestine-digesta samples, pond water, sediment and feed. For this, a denaturing gradient acrylamide gel was prepared by mixing a low (30%) and high (60%) gradient solution made up of urea and formamide. From each sample, 45 µl of the amplified 16S rRNA V3 region template was loaded in the gel and run for nearly 14 hours at 60 V/cm and 60oC to maintain the denaturing conditions. After that the gel was washed, stained using silver staining protocol and bacterial diversity was visualised.

Though phylogenetic identification of bacterial diversity was not possible, we were able to derive certain salient observations from the DGGE study. As observed in the bacterial count, bacterial diversity in the fish gut was higher than in the environment (see the number of bands). While some of the gut microbes (bands 3 and 4) were sourced from the environment (pond water, sediment and feed), others were apparently unique to the fish intestine and digesta (bands 2, 5, 6 and 7). Based on the band pattern differences between the digesta and intestine samples, we can say that not all bacteria (band 4 and 7) are able to colonise the intestine of Indian major carps. But at the same time, there are groups which are efficient (band 6) in colonising the intestine. This is a clear indication for the presence of resident and transient forms of gut bacteria in major carps, with a possible core group. In the intestine samples, the difference in band intensities (band 1, 2 and 3) and banding pattern suggests the possible host-specificity of gut microbes in Indian major carps. Overall, our preliminary culture-independent observation provides few notional answers for the questions concerning the gut microbiota of polycultured Indian major carps and there are similarities between our culture-dependent and independent observations. But, still we need to carry out high-throughput NGS investigations on major carp gut microbiome to characterise the phylogenetic diversity and host-specific dynamics.

Prospects and applications

Continuous advancement in culture-independent high-throughput technologies are opening the doors to a microbial world which is far beyond our expectations and we are just starting to understand the great diversity of gut microbiome and the way they shape animal biology. In Indian major carp polyculture systems, first, we need to generate a comprehensive understanding of gut bacterial diversity and community

structure, with which we can assemble a clear picture of host-microbe and microbe-microbe interactions. Based on this foundation, we can then probe the factors that influence the gut microbiome phylogenetics and their metagenomic functional roles in fish nutrition, metabolism, growth and health. Ultimately, using this understanding of the intricate host-microbe symbioses and core microbiome functions in healthy conditions, we can monitor husbandry conditions in farms and manipulate gut microbes to decrease disease susceptibility and increase feed efficiency/productivity. Also, we can relate the carp gut microbes to many other production aspects such as changes in nutritional requirements, environmental adaptation, microbial spoilage and antibiotic resistance. In the long run, we need to look beyond bacterial communities and explore yeast, virus, archaea and protozoan populations that are functionally associated with fish gut microbiome.

Acknowledgements

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Figure 5: PCR-DGGE gel showing bacterial diversity of the samples.

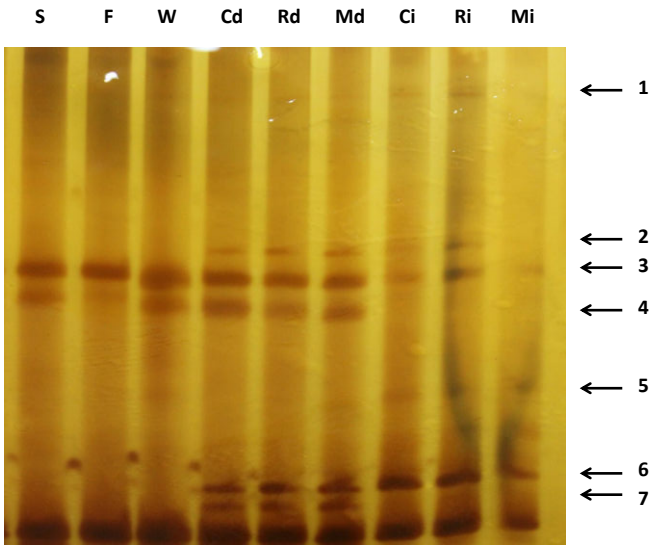
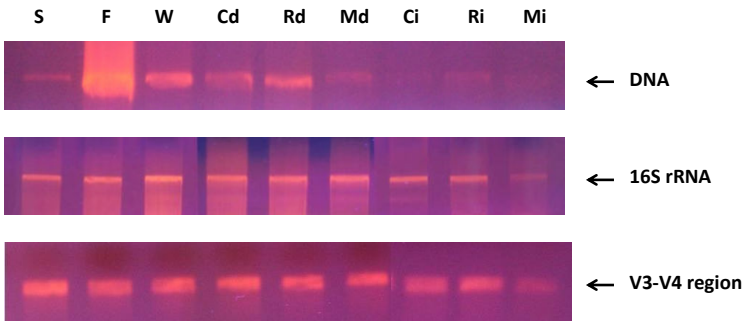


Figure 4: Extracted genomic DNA and amplified bacterial 16S rRNA gene and V3-V4 region.



S, sediment; **F**, feed; **W**, water; **Cd**, catla digesta; **Rd**, rohu digesta; **Md**, mrigal digesta; **Ci**, catla intestine; **Ri**, rohu intestine; **Mi**, mrigal intestine

Integrated rice-fish farming in hilly terraces of the Apatani Plateau, Arunachal Pradesh

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The wet rice terraces of Apatani plateau.

Arunachal Pradesh is the largest of the northeast Indian states, situated on the extreme north-eastern tip of India in the trans-Himalayan region. The state has a population of 1.38 million (2011), comprising a mosaic of cultures and traditions, with 28 major tribes and 110 sub-tribes. The land is the richest biotic prefecture of India due to its altitudinal variations and distinctive weather and climatic conditions, which are mostly dominated by the Himalayan system.

Due to the advantageous ecological conditions, agriculture is the main source of earnings in Arunachal Pradesh. Among the important crops grown in the region viz., maize, millet, wheat, pulses, potato, oil seeds, fruits and vegetables, rice is the most congenial as the major crop. These rice fields in turn have an immense potential to augment fish production in the state by providing refuges for fish. This technique of rice-fish farming is most popular among the Apatani tribe in the Ziro valley of Arunachal Pradesh in their wet rice terraces. The strains of fish species include *Cyprinus carpio specularis* (mirror carp), *C. carpio communis* (scale carp) and *C. carpio nudus* (leather carp) cultivated synchronously with local rice cultivars *(Oryza sativa)*, viz. e*amo, ampu, mipya, pyapu, pyaping* and *eylang.* The present communication is based on the information gathered by the authors during a field visit to Ziro valley for research and development programmes at villages of Yachuli, Hapoli, Hong, Hari, Hija, Bula, Dutta and Old Ziro since 2016. We interacted with the rice-fish farmers, village headmen, state fishery officers and the scientific staff of KVK to understand the skills of farming, collect samples, and learn the local socio-economic circumstances and culture in developing this manuscript.

The Apatani Plateau

The Apatani Plateau of Ziro valley is situated at around 1,550 meters altitude. The entire valley is bestowed with a unique and ingenious integrated rice-fish farming practice, locally called *ajii-ngyii*. This form of agriculture occupies around 59% of the total land area and is surrounded by hill forest (14%) interspersed with bamboo and pine agroforestry systems (17%). The land use and land cover map (Table 1) developed from GIS analysis shows a total area of 4,100 hectares as productive in the Ziro valley. The valley is split by the Kiley River and flows between the river valleys of Kamla on the north, Panior in the south and Pein on the east. All of these rivers eventually drain into the Subansiri, a major tributary of the Brahmaputra River in its north bank. The plateau receives a mean rainfall of about 400 mm, mainly during May-August and relatively little or no rains during November-February. The temperature remains mild to warm in summer and cold in winter. The annual temperature in the valley ranges from 5°C - 30°C. The wide temperature range is conducive for growth of both warm and coldwater fisheries. The cool summer weather makes the valley a major destination for

tourists all around the world. Unlike other states of India, there are neither any professional fishers in the state nor a community that can be designated as a fishing community. Thus, fish farming has been practiced on traditional lines and this knowledge has been passed on through the generations. A few of the villages where one can experience this age-old practice in the plateau are Hong, Hari, Hija, Bula, Dutta, Mudang, Bamin and Old Ziro. Hapoli is the headquarters of the Ziro valley or the Apatani Plateau with the established local administration, schools, banks and most essentially to this text the markets.

The integration of rice-fish in terraces

The people of the Ziro valley are known as *'Apatanis'* and henceforth the name Apatani plateau. They are mostly agrarian in habit and possess a rich traditional knowledge in farming practices. The efficient water management and sustainable use of the agricultural land and waste products for integrated wet rice-fish cultivation *(ajii-ngyii)* by these Apatanis in the plateau remains unmatched if compared to other tribes of the north-eastern states. Women play a major role in rice-fish cultivation from land preparation to marketing of the farm produce.

Preparation of rice-fish terraces

The rice-fish terraces are prepared soon after the final harvest of rice in the month of November. The paddy stem is cut in its mid part during the harvest of rice and the left over stems are allowed to remain in the field to decompose. The water from the terraces is completely drained so that the soil bottom is exposed to sun and dried until it cracks. This is done to avoid crop loss from pests present in the soil. During

Preparation of terraces & trenches for rice fish farming.

December-January, the farmers start plowing their fields with economically and ecologically viable conventional chopping implements *(daos)* and spades. The farmers neither make use of animal power, machines nor any advanced tools to plow their fields. This is a traditional practice and emphasis is essentially given to three major components of land preparation (Baruah and Singh, 2018) viz., the strengthening of dykes *(agher)*, the channelisation of irrigated water in the fields and the digging of trenches *(siikho/parkho/hehte)*. The earthen dykes are repaired every year by strengthening the top soil and raising the dykes up to 40-70 cm height. A freeboard of 15-25 cm is kept along the height of the dyke and a width of 30-70 cm is maintained, which is efficiently used for the production of various vegetables such as cucumber, brinjal, tomato, pumpkin, chilies, beans and crops such finger millets and maize. Raising crops on the dykes also prevents erosion of soil in water filled terraces. The irrigation system in wet

Raising of additional crops on the dykes of rice fish terraces.

A feeder channel to the rice fields.

rice terraces is unique in the valley. The system is comprised of a primary channel connected to the main Kiley River. The primary channel in turn drains its water to a highly webbed feeder channel system so that each feeder channel can at least be linked to one of the rice-fish terrace in the plateau. The feeder channels not only optimise the usage of water but also provide nutrient wash-out to the paddy field from the adjoining catchment areas. The water is conveyed from one terrace to another through a bamboo, wooden or galvanised iron pipe installed at a height of 15-25 cm above the bed to ensure proper water level. In order to contain soil erosion, bio-fencing is installed alongside of the primary channels. The trenches (30-45 cm depth) are most distinctively dug within the rice-fish terraces to facilitate movement and provide refuge to fish during warmer hours of the day. The trenches *(siikho/parkho/hehte)* are either dug perpendicular to one another or at times irregularly webbed. The trenches occupy 8-12% of the total area in each of rice-fish terraces. The trenches are provided with two outlets *(hubur)* one at the surface to release excess water and the other at the bottom for draining the field water for harvesting the fish. Both outlets are strictly guarded with bamboo screens to prevent escape of fishes during the culture period. A water level of 25-35 cm is maintained in the rice-fish plots during the season. The soil nutrients of these terraces are also retained in the plots by incorporating organic manures in the form of dung from cows and pigs and poultry litter. Azolla *(tapang)* are naturally grown as nitrogen fixer (Liu, 1995) during the fallow period and are mixed with soil at the time of field preparation. Similarly, *Lemna* spp. *(murta tapang)* is also seen growing in the rice terraces which might serve as fish food as well as a source of organic compost after its decomposition. Other inputs provided in these fields are self-decomposed agricultural wastes, paddy straw, rice husk, ash, weeds and similar materials. Subsequent to harvest, cattle are also grazed in the plots to add manure. Farmers also reported that the litter of decomposed leaves which reach out from the adjoining forest land are collected in separate water channels and are later drained to one of the primary channels to reach the cultivated plots. Treating these terraces with manures enhances the soil productivity on one hand and aids in

Natural fertilisation of the rice fish terraces with azolla and lemna.

Nursery beds for rice transplantation.

natural fish food production on the other hand. Nursery beds *(midding)* are simultaneously prepared near to these terraces on a slightly elevated surface so as to maintain an optimum quantity of water, not allowing the beds to get too dry or too wet. The nursery beds are well protected with bio fencing to avoid grazing stray animals. The seed for these nurseries are prepared in the previous year and utmost care is taken to preserve it for better germination. The rice seeds are sown in the month of February-March in these nurseries for later transplantation in the main fields.

The rice-fish-horticulture system

Efficient water management utilising water from natural streams in well planned manner is the basis for making the Apatani system suitable for rice and fish cultivation together. Women play an important role and share their work equally with men in rice-fish cultivation. Fifteen varieties of the local rice (*Oryza sativa)* are reported to be cultivated in the Ziro valley which is mostly categorised into three cultivars *eamo, mipya* and *pyaping* (Kacha, 2016). Transplantation of rice seedlings from the nurseries to the prepared terraces is conducted in April. The 65-75 day old seedlings of 10-12 cm in height are sowed manually *(aemo lilo)* by hand, keeping a distance of 4-8 cm between each. Timely weeding and partial transplanting over dead seedlings are done periodically. Finger millet *(Eleusine coracana)*, soya beans, buckwheat, maize, and barley are grown on the dykes as additional crops (Fig. 9). Vegetables such as cucumber, brinjal, tomato, pumpkin, chilies and radish are also grown on the dykes. All these crops are sown in April-May using a wooden dibbler *(damu)*. Fruits such as kiwi are grown in the adjoining lands if not directly over the dykes. Successive weeding *(ahru-hodo)* in rice field and dykes is carried out by manual labour using artisanal tools during the growing season.

Fish rearing in the fields involves one batch or two batches in a year depending upon terrace conditions. The most favoured fish species are the strains of common carp, scientifically known as *Cyprinus carpio specularis* (mirror carp), *C. carpio communis* (scale carp) and *C. carpio nudus* (leather carp) for integration with local rice varieties. Young fish of 5-8 cm size are stocked in the rice terraces 10-15 days after transplantation of rice seedlings, preferably during April-May.

Chaudhary et al. (1993) advocated rice-fish systems as an ideal integration in any rice ecosystem having fertile water, even with lower depth.The fish feed on small insects such as water beetle and larvae, which are harmful to the paddy. The waste material voided by fish acts as fertiliser for the rice. Additionally, the browsing habit of some fish such as common carp help to release fixed nutrients from the soil, also improving rice productivity (Halwart and Gupta, 2004). Apart from the common carp, species such as grass carp *(Ctenopharyngodon idella)*, silver carp *(Hypophthalmichthys molitrix)*, *Barbonymus gonionotus*, *Labeo gonius* and other

Table 1: Land use land cover of the Ziro valley

Category	Area (ha)	(%)
Agroforestry	684.34	16.69
Aquaculture	1.64	0.04
Built up (rural)	92.20	2.25
Built up (urban)	117.70	2.87
Forest	577.82	14.09
Rice fish cultivation	2423.88	59.12
Shifting cultivation - abandoned	115.10	2.81
Shifting cultivation - current	4.72	0.12
Transportation	60.29	1.47
River / stream / drain	22.31	0.54
Total area	**4,100.00**	**100.00**

Labeo species are also stocked in the plots. The fish feed on plankton and periphyton (Saikia and Das, 2008) from the system, reducing the need for expensive, externally supplied feeds. Das et al. (2007) recognised the system as a self-subtracting periphyton-based aquaculture system for the role played by rice as a surface for periphyton growth. Among the different fish species cultivated, the best results in terms of fish growth and survival is observed in common carp varieties due to their robust and hardy nature. During weeding in the rice cultivated areas fishes are guided to the dugout trenches. In the event of low rainfall and hot weather, the stagnant water of the open field becomes warm and the water in the deep trenches provides cool hideouts for the fish.

The rice-fish environment

The wet rice field is described as a temporary aquatic environment by Roger (1996) or a special type of wetland that can be considered a successor of shallow marshes or swamps by Ali (1998), which is influenced and maintained by farmers' activities. Wet rice terraces of the Apatanis may be categorised under irrigated rice ecosystems as classified for agro-ecological zones by IRRI (1993). These terraces have a perennial supply of water which has high potential for rice-fish cultivation. The aquatic environment in rice terraces of the Apatani plateau is heavily influenced by water flow in the connecting feeder channels. Typical abiotic parameters of these water channels and rice-fish environments are summarised in Table 2. Likewise, organic inputs in these rice-fish terraces increase the growth of both phytoplankton and zooplankton diversity.

Owing to the nature of the water, the aquatic flora and fauna in rice-fish environments have their origins in the irrigation channels and river sources. In the present study, it was observed that the rice-fish plots are rich in phytoplankton viz., *Spirogyra* (12-47%), *Oocytis* (40%), *Navicula* (5-14%), *Pinnularia* (6-13%), *Nitzschia* (13%), *Ulothrix* (13%), *Closterium* (13%), *Stigeoclonium* (11%) and *Ankyra* (7%). The zooplankton studies revealed that the copepods (11-90%) dominated the rice fish plots followed by cladocerans (5-25%). Similarly, the connecting feeder canals are dominated by phytoplankton such as *Fragilaria* (55%), *Spirogyra* (24%), *Spirulina* (12%), *Nitzschia* (11%), *Navicula* (7%) and *Oscillatoria* (2%). In case of fish ponds, *Euglena* dominated the phytoplankton (63%), followed by *Nitzschia* (17%), *Navicula* (14%), *Stephanodiscus* (10%) and *Desmidium* (2%). Zooplankton composition was not recorded.

The fish seed availability and hatchery facilities

The Apatani plateau is a hill locked area and is devoid of much infrastructure for fish seed production, such as a fish hatchery operated on scientific guidelines. However, some amount of common carp seed is produced by limited farmers of the valley which is not sufficient enough to fulfil the demands of the entire region. Therefore, fish seeds are required to be procured from the neighbouring state Assam in huge quantity. Fish seed vendors from Assam carry the fry and fingerling sized fish seeds to the valley and stock them in small ponds and tanks along the roadside. But the import of fish seed from other states incurs a high transportation

Table 2

Physico-chemical parameters	Water resources			
	Rice fields	**Feeder canals**	**Fish ponds**	**River**
Dissolved oxygen (mg/l)	8.92±0.67	8.97±0.34	8.27±0.21	8.97±0.32
pH	5.69±0.21	5.81±0.20	6.12±0.54	5.75±0.16
Temperature (°C)	23.99±1.30	23.21±0.74	26.58±1.61	21.31±0.19
Total dissolved solids (mg/l)	7.17±3.54	11.00±6.29	34.00±28.48	7.33±0.58
Salinity (mg/l)	0.005±0.005	0.01±0.01	0.02±0.01	0.01±0.00
Atmospheric pressure (psi)	839.48±1.90	837.17±3.46	849.77±20.64	840.00±0.87
Resistivity (MΩ-cm)	0.09±0.04	0.06±0.03	0.03±.002	0.067±0.00
Conductivity (µS/cm)	13.83±6.74	21.67±12.85	68.33±57.18	17.33±3.21
ORP (mV)	103.02±47.63	70.40±11.50	130.63±90.66	78.60±2.46
	Nutrients			
Ammonia (mg/l)	1.04±1.03	0.51±0.83	0.34±0.57	0.51±0.10
Nitrate (mg/l)	1.87±1.33	1.42±0.66	2.00±1.00	1.13±0.63
Nitrite (mg/l)	0.03±0.03	0.07±0.05	0.03±0.02	0.03±0.02
Hydrogen sulphide (mg/l)	0.29±0.12	0.35±0.14	0.27±0.15	0.15±0.06
Alkalinity (mg/l)	63.75±21.34	43.33±16.33	43.33±40.41	42.50±17.08
Total hardness (mg/l)	26±19.18	46.00±12.52	52.00±13.86	29.00±8.87
Phosphate (mg/l)	0.62±0.35	0.42±0.49	0.67±0.58	0.38±0.48
Fluoride (ppm)	0.23±0.22	0.39±0.19	0.50±0.00	0.21±0.05
Residual (free) chlorine (mg/l)	0.02±0.01	0.02±0.01	0.02±0.01	0.01±0.01
Iron (mg/l)	0.73±0.79	0.53±0.73	1.50±0.87	0.31±0.15
Silica (mg/l)	2.50±2.67	3.33±2.58	3.33±2.87	2.50±2.89

Above: Raising of additional crops on the dykes of rice-fish terraces. Below: Plantation of kiwi fruit adjoining fish ponds.

Stocking of juvenile fishes in the rice-fish plots of Ziro valley.

cost, and in turn raises the price of fish seed. Observing this constraint, ICAR-DCFR, Bhimtal, took initiatives in establishing a portable FRP made fish hatchery unit at Hari Village during 2018 in association with the Department of Fisheries, Government of Arunachal Pradesh and with the participation of the Apatani community under the banner of the Gaumco Multipurpose Cooperative Society. Members of the society were given hands-on training in broodstock management, hatchery operation, fish seed production, transportation and marketing by exposing them with the fundamental functionalities of a recognised fish hatchery at Pabhoi Fish Farm, Biswanath, Assam in the same year. This exposure visit helped the society members to undertake the fish breeding programmes, hatchery operation and fish seed production on their own. Furthermore, earthen nurseries were also developed within the premises of the FRP fish hatchery, enabling the farmers to stock different sizes and species of fish seed produced from the hatchery unit. The hatchery is being operated by women members of the society. The sustainable production of fish seed for stocking in rice-fish farming will expand women's participation further in the region.

Harvesting from rice fish terraces

The crop of rice is harvested (*antee pila* or *antee dandu*) during September-October based on the time of sowing of the rice seedlings. Rice grains are collected in a bamboo basket on the field by thrashing the grains from the stalk. The collected grains are then transported in these baskets to the granaries built by the farmers in their villages. The production rate is around 500 kg/ha/season (Saikia and Das 2008). But during our investigation it was found that the rice production may reach up to 10 tonnes/ha/season in the rice-fish terraces.

Below: Lateral dugout trenches provide hide outs for fishes.

Above: Imported fish seed from Assam. Below: Establishment of a fish hatchery at Hari village.

Skill development in fish seed production for fish farmers of the Apatani plateau.

Gear constructed from bamboo, to collect the harvested fishes from terraces.

Additional crops sowed on the dykes such as finger millet, soyabean and maize are harvested during August-September. The vegetables are harvested from time to time during July-October. For harvesting of the fishes, water is completely drained out from the paddy field. This compels the fishes to concentrate in the trenches from where they are caught by hand or by using the traditional bamboo and cane woven gears. The fishes are completely harvested before harvesting rice from the terraces. Common carp generally gain weight up to 300-500 g within a span of 3-4 months as reported by earlier investigators. However, it was observed during our field survey that the farmers start selling the fishes when they attains a weight of 65-80 g. The harvested fishes are cleaned in fresh water and are transported to the fish market.

Marketing and usage of farm produce

The varieties of rice are mostly used for self-consumption as rice is the staple food for the people of Ziro valley. Finger millet is used for food in the form of flour and for the preparation of local wine *(sarse-o)*. Similarly, maize, soyabean, buckwheat and barley are also used as local food and some of them for preparation of wine. Vegetables are sold in the market at varying prices ranged from INR 20-80. The harvested fish are carried to the local market at Hapoli, the district headquarters in Ziro valley in live condition. This short distant transportation is facilitated by carrying the live fishes in finely woven bamboo and cane cone-shaped baskets (*ajii piiwa* or *ajii raju*). The fishes are packed in layers. The basket measures approximately 50-70 cm in height and 30-45 cm at its mouth circumference and 25-35 cm at its base circumference. The transported fishes are immediately released in water filled trays in the market. The trays are made of finely woven bamboo and cane material on which a polyliner is spread to retain the water and to keep the fishes alive. The live fishes are sold at a rate of INR 300 per kg (2018). This price is fetched over the initial cost of INR 1-5 per fingerling at the time of stocking. Thus the net profit for the farmer stands more than 100% earning in addition to their regular crop of rice.

Conclusion

This study on terrace farming in the Apatani Plateau reveals that the integration of rice with fish is a low-cost sustainable practice for the rural masses to obtain high value protein, nutritional security and income from a unit area. Rice-fish farming reduces the usage of fertiliser, pesticides and herbicides in the rice field and with zero input of artificial feed to fish. Such reduction of input costs lowers farmer's economic load and increases their income from fish sale. Having such additional income, the net productivity from rice-fish integrated farming is observed to be much higher than monoculture of rice alone in the valley. Therefore, this low input self-supporting system of traditional rice-fish culture of Apatani plateau can be very well extended as a farmer-friendly avenue in other parts of Arunachal Pradesh, with necessary location-specific refinements. The quality of water is the biggest factor in any rice-fish farming system and are subject to the source of water from the feeder canals and connecting rivers. The abiotic parameters of water in rice-fish terraces of the plateau are within the optimum range and conducive for rice-fish farming. In addition, establishment of a private fish seed hatchery in the Ziro valley, along with a few maintained fish brood banks is an added advantage for ready availability of quality and quantity fish seeds for self-sufficiency among the rice-fish growers. Trials have also been made to upgrade the present strains of common carp to high yielding strains for faster growth and better productivity. The participation of women is an integral part of this farming practice and empowering them with skills in fish seed production will further enhance the farm productivity, and promote ancillary business such as net preparation, mending, feed supply and fish seed trade.

Above: Harvesting of finger millet from rice-fish dykes. Below: Fish harvested from the terraces.

Fish harvested from rice-fish terraces.

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Live fish market at Hapoli, Ziro valley.

Above: Harvesting rice in the Apatani plateau. Below: A market of Ziro valley with locally produced fish and vegetables.

NACA Newsletter

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30th NACA Governing Council, China

Participants in the 30th Governing Council Meeting.

The 30th Governing Council was held in Guangzhou, China, 26-27 March. 74 participants attended the Governing Council Meeting representing:

- Fifteen member governments, and the Kingdom of Saudi Arabia.
- The Regional Lead Centres from China, India, the Philippines and Thailand.
- The Food and Agriculture Organization of the United Nations.
- The Southeast Asian Fisheries Development Centre.
- The Network of Aquaculture Centres in Central and Eastern Europe.
- The Hungarian Research Institute for Fisheries and Aquaculture.

The opening ceremony featured speeches from the Chinese Government and academia. Mr Liu Xin Zhong, Deputy Director General, Fisheries Administration Bureau, Ministry of Rural Affairs, gave the opening speech. Mr Gu XingWei, Director General, Guandong Department of Agriculture and Rural Affairs, gave a welcoming address. Dr Liu YingJie, Vice President of the Chinese Academy of Fisheries Sciences, gave remarks on the role international collaboration had played in regional aquaculture development.

The outgoing Chair of the Governing Council, Dr Shakeel Hassan, welcomed delegates and thanked the Government of China for hosting the meeting. The Director General of NACA, Dr Cherdsak Virapat, reflected on the role of networking, sharing and learning for food production, economic growth and livelihoods.

The host Government, China, was elected as Chair of the 30th Governing Council. Hong Kong SAR was elected as Vice Chair. Highlights of the meeting included:

- The election of Dr Huang Jie as the next Director General of NACA.
- The Kingdom of Saudi Arabia attended the Governing Council for the first time, as an observer. Saudi Arabia wishes to strengthen cooperation with the network in aquaculture development. The Kingdom presented an overview of the status of national aquaculture development.
- Presentations on recent activities undertaken by the NACA regional lead centres. Aquatic animal genetic resources, health and biosecurity featured as issues of common interest. Of particular concern, the use of antimicrobial substances in aquaculture and antimicrobial resistance.
- Preparations for convening the next Global Conference on Aquaculture Development. The conference will be held in China, in late 2020.

Dr Huang Jie elected as next Director General of NACA

NACA welcomes Dr Huang Jie as the incoming Director General of NACA. He will serve a five year term beginning in May 2019. Dr Huang succeeds Dr Cherdsak Virapat, who will complete his own five-year term in April. Dr Huang was elected at the 30th Governing Council Meeting held 26-27 March in Guangzhou, China.

Dr Huang, a Chinese national, obtained his BSc on virology in Wuhan University in 1987, an MSc in the Wuhan Virology Institute, Chinese Academy of Science (CAS) in 1990, and his PhD on marine biology in the Ocean Institute, CAS, in 2010.

He is a Senior Researcher of the Maricultural Disease Control and Molecular Pathology Laboratory, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (CAFS); the Chief Scientist of CAFS on aquatic animal disease control; an OIE Designated Expert for White spot disease (WSD) and Infectious and haematopoietic necrosis (IHHN); and a doctorial tutor for Shanghai Ocean University.

Dr Huang has been conducting research projects on the diagnostics, epidemiology, molecular mechanism of virus infection and control technology for WSD and other aquatic animal diseases for 26 years. He identified a new virus, HHNBV (previous named WSSV), as the pathogen of WSD in China in 1993 and reported the transmission route of the virus. His research group has discovered several new viruses, new genotypes, or new emerging diseases in marine farming industries of China, including turbot reddish body iridovirus; acute viral necrotic virus in scallop; covert mortality nodavirus in shrimp; a new genotype of yellow head virus (YHV-8) in shrimp; an earliest identified virulent strain of *Vibrio parahaemolyticus* in shrimp causing acute hepatopancreatic necrosis disease (AHPND) in 2010; shrimp hemocyte iridescent virus (SHIV), and a virulent strain of *V. campbellii* causing AHPND.

His laboratory has established a series of detection techniques, including gene probes, PCR, LAMP, and gene chips, for different aquatic animal pathogens and national standards for shrimp diseases diagnosis. They have also developed rapid detection kits for more than 20 aquatic animal pathogens, non-specific immunoenhanceants and probiotic bacteria for shrimp disease prevention, microorganism-enhanced biofloc technology for aquaculture, and marine fish vaccines for *V. anguillarum* and *Edwardsiella tarda*. Dr Huang proposes the concept of microbiological control technology to prevent aquatic animal disease and actively promotes the concept of biosecurity systems for the aquaculture industry.

Dr Huang has more than 330 publications of which 80 were published on international journals, has obtained 48 patents, published 30 national or professional standards, won 13 national and provincial awards, and trained 94 doctoral and masters level students. He won the Distinguished Expert for TAISHAN scholars of Shandong Province, the Excellence Talent and Innovation Team for Agriculture Research, and holds other national, provincial and ministerial honor titles.

Expert Consultation on Genetically Responsible Aquaculture

Participants in the Expert Consultation on Genetically Responsible Aquaculture.

A Regional Expert Consultation on Genetically Responsible Aquaculture was convened by the ICAR National Bureau of Fish Genetic Resources, India, from 26-27 February. The consultation was co-organised with NACA. 36 experts attended from throughout the region.

The consultation discussed the role of:

- Certification and standards for quality seed production.
- The development of field-validated protocols for testing the origin, genetic composition and inbreeding of seed.

- Intellectual property rights.
- Materials transfer agreements.
- Verification of origin though the use of molecular markers.
- Safeguarding farmed stocks from genetic erosion.

The immediate objective of the consultation was to discuss mechanisms for establishing quality seed production systems to improve hatchery and on-farm genetic diversity. Such systems are envisaged to include verified seed and brood-stock, biosecure procedures for germplasm exchange, and quantifiable standards to empower farmers and regulators.

A long-range objective is to establish networks of registered, small broodstock holdings. Linked via IT systems, the networks will form a virtual global aquaculture gene pool that can, collectively, sustain high genetic diversity, environmental resilience and long-term capacity for adaptation, while checking inbreeding depression.

There is a widespread perception that inbreeding and genetic erosion is leading to a decline in productivity in aquaculture. However, it is difficult to separate the impact of genetic erosion from that of other factors such as poor husbandry, disease and environmental issues. To date, the evidence has largely remained anecdotal.

The use of genetically improved or specific pathogen free varieties in aquaculture is relatively uncommon, compared to terrestrial livestock industries. Animal breeders have few rights, compared to plant breeders. There are presently no convenient and validated assays or standards available for testing genetic composition and inbreeding.

The issue is complicated by the unauthorised practice of “copying” or multiplying improved seed by third parties, without technical supervision. This may not only reduce performance but may also increase susceptibility to disease, particularly in shrimp.

The development of standards and certification processes, protocols for assessing inbreeding and origin of seed, and improved frameworks for managing intellectual property rights are expected to help bring aquaculture up to speed with other livestock sectors.

Patrons of the consultation were Dr Trilochan Mahapatra, Director General, ICAR and Secretary, DARE; Dr J.K. Jena, Deputy Director General, ICAR; and Dr Cherdsak Virapat, DG, NACA. The consultation was convened by Dr Kuldeep Lal, Director, NBFGR and Dr Roger Doyle, President of Genetic Computation Ltd. FAO was represented by Dr Graham Mair.

www.enaca.org

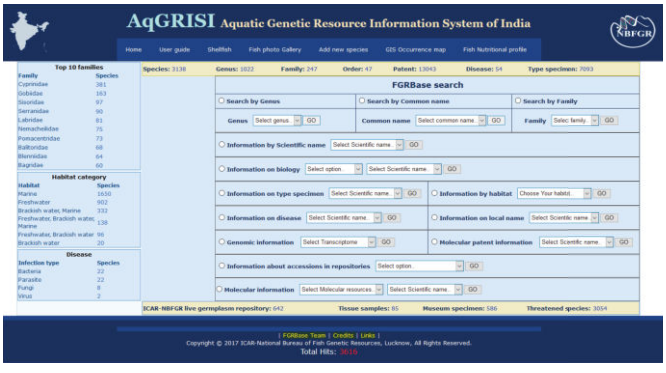
Launch of AGRISI: Aquatic Genetic Resource Information System of India

AGRISI, a new information system on aquatic genetic resources of India, has been launched by the National Bureau of Fish Genetic Resources (NBFGR).

AGRISI is a unique platform presently covering 3,138 native fish species of India. The system provides information on systematics, biology, distribution, nutrition, nutrition, and other characteristics.

AGRISI includes information on museum specimens including type specimens, and accessions from different NBFGR repositories including data on germplasm and cell lines. AGRISI links to other molecular resources developed under the National Agricultural Bioinformatics Grid. These include:

- FBIS, the Fish Barcode Information System.
- HRGFish, a database of hypoxia responsive genes.
- FishKaryome, a chromosome database of fishes and other aquatic organisms.
- FishMicrosat, a fish and shellfish microsatellite database.
- FMiR, fish mitogenome resources.



AGRISI was developed under the Digital India Initiative Programme. It provides country-specific information on fish genetic resources as required by the BDA Act, 2002. It also supports FAO’s Report on the State of the World’s Aquatic Genetic Resources for Food and Agriculture.

The database is accessible at: http://mail.nbfgr.res.in/agrisi/

Aquatic animal epidemiology training course held at NBFGR

The ICAR-NACA School on Aquatic Epidemiology and Disease Surveillance was held at the ICAR National Bureau of Fish Genetic Resources (NBFGR) from 1-6 March. The school was a collaboration between the Indian Council of Agricultural Research and NACA.

Participants were welcomed by Dr Kuldeep K. Lal, Director of NBFGR. Dr Gaurav Rathore, Head of the Fish Health Management and Exotics Division introduced the programme.

The course was lead by Professor Kenton Morgan, Ex-Chair of Epidemiology at the University of Liverpool. Dr Eduardo Leano, Coordinator, Aquatic Animal Health Programme, NACA and Dr I. Karunasagar, ex-FAO, gave invited lectures.

The school covered:

- Concepts and principles of epidemiology.
- Use of epidemiological principles in design and implementation of surveillance programmes.
- Sampling considerations for surveillance programmes.
- Population surveys.
- Estimation of sensitivity and specificity of diagnostic tests.
- Questionnaire design.

NACA would like to thank the ICAR-National Bureau of Fish Genetic Resources, its staff and Professor Morgan for their initiative and collaboration.

Asia-Pacific Laboratory Proficiency Testing Workshop

Participants in the Asia-Pacific Laboratory Proficiency Testing Workshop.

The Australian Government is conducting a new aquatic animal disease proficiency testing programme. The programme provides laboratories with the opportunity to assess their own diagnostic performance. This allows them to identify technical issues with their practices and improve their performance.

34 laboratories from thirteen countries in the region are participating in the programme. The programme involves eight rounds of proficiency testing carried out over four years. Diagnostic performance is assessed against ten priority fish and crustacean diseases. The first two rounds of samples were distributed for analysis in 2018.

The programme convened a proficiency testing workshop from 13-14 March in Bangkok, Thailand. The aim was to improve the performance of laboratories and technical personnel, by:

- Providing an opportunity to discuss experience with the first two rounds of testing.
- Improving personnel's understanding of diagnostic standards, proficiency testing procedures and laboratory accreditation.
- Identifying current capability and future training needs to meet international trade requirements.

The workshop was convened by the Department of Agriculture and Water Resources and CSIRO's Australian Animal Health Laboratory (AAHL), in collaboration with NACA.

The programme builds on previous laboratory proficiency testing exercises held in 2012. These exercises, also supported by the Australian Government, significantly improved diagnostic performance in participating laboratories.

The workshop included presentations and discussions on:

- The importance of accurate diagnostics for aquatic animal disease to facilitate trade.
- Quality assurance management systems and assay validation.
- Australia's laboratory network and aquatic animal disease surveillance and diagnostic activities.
- PCR laboratory design and laboratory workflows.
- Sample preparation and extraction.
- Trouble shooting and discussion on test performance.
- Method validation.
- OIE Aquatic Manual update.

- Method implementation and equivalence testing.
- Quality assurance and laboratory accreditation.
- Presentations on WSSV, YHV1 and MCV testing.
- An overview of the WSSV outbreak and national surveillance in Australia.

NACA would like to thank the Australian Department of Agriculture and Water Resources and CSIRO/AAHL and for their generous support.

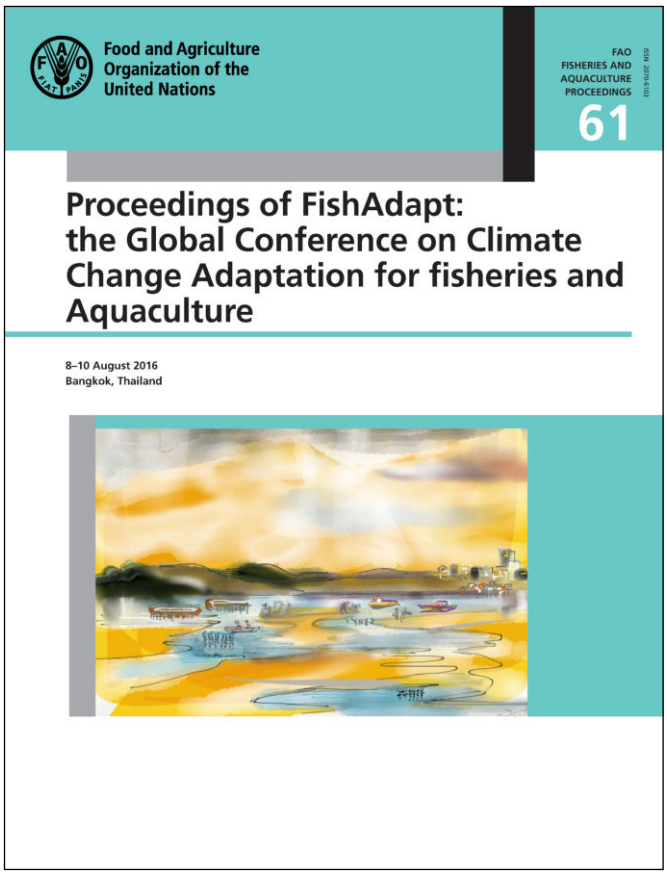
Proceedings of the FishAdapt Conference

Climate variability and change are affecting hydro-meteorological cycles and altering aquatic ecosystems, driving shifts in physical and chemical processes, ecological communities and the distribution and abundance of species. These changes have implications for fisheries management, food security and the livelihoods of more than 600 million people worldwide that are employed in fisheries and aquaculture, their value chains and related industries.

The FishAdapt conference was held in Bangkok from 8 to 10 August, 2016. It provided a forum for scientists, development professionals and natural resource managers working in the context of fisheries, aquaculture, rural development and related fields to share practical experiences in understanding the vulnerabilities associated with climate change and ocean acidification and the development of risk management and adaptation strategies. The conference bridged interdisciplinary gaps and provide a wider, shared perspective on the issues and the current state of knowledge.

The proceedings of the conference share the experiences of the 110 participants from 27 countries and show that much can be done at the household, community and sector levels to support the resilience of the sector and its dependent communities in a changing climate. Download from:

https://enaca.org/enclosure.php?id=1039



Quarterly Aquatic Animal Disease Report, July-September 2018

The 79th edition of the Quarterly Aquatic Animal Disease report contains information from eleven governments.

The foreword discusses the outcomes of the 17th Meeting of the Asia Regional Advisory Group on Aquatic Animal Health, held in Bangkok, 13-14 November 2018. Free download from:

https://enaca.org/?id=1036

Centex Shrimp: International Training Course on Biology and Pathology of Penaeid Shrimp

This year's course will take place from 1-12 July, Thailand. Tailored to those interested in doing shrimp research or learning about shrimp diseases, you will get to learn from the very best in the field about major and emerging shrimp diseases, shrimp farm management, gross inspection and molecular diagnosis of shrimp infectious diseases. You will have opportunities to try your hands in a series of practical sessions, including anatomical inspection using digital slides, nucleic acid detection, EHP spore purification and detection, and many more.

For more details, please email sccentexshrimp@mahidol.ac.th.

INFOFISH World Shrimp Trade Conference and Exposition

The theme for Shrimp 2019 is “modelling for sustainability”. The conference will be held from 12-14 November in Bangkok, Thailand.

Global production of farmed shrimp is estimated at between 2.9 million MT and 3.5 million MT, with Asian producers – China, India, Indonesia, Philippines, Thailand and Vietnam – sharing 75-85% of the total volume.

Following a moderate recovery in the sector in Thailand and Mexico, and expanded vannamei farming focused in China, India, Indonesia and Vietnam, overall production is expected to be positive in 2018.

Driven by environmental and social factors, as well as the recurrent disease outbreaks in shrimp farming, the focus now is towards sustainability.

With the various challenges faced by the shrimp industry, there is a need to properly identify and promote systems that will lead to sustainable development. Knowledge of credible standards and regulations of different importing/exporting countries is of paramount importance to facilitate compliance and harmonious trade.

Further, opportunities to strengthen international co-operation on technical, policy and trade issues on global shrimp industry must be highlighted.

The conference

The international conference on shrimp will consider at length the present and future of the industry, focusing on moving towards sustainability.

A large part of the sessions will be devoted to production and trade at the domestic and international levels, as well as the latest developments in the shrimp industry.

Some 250 delegates, both international and local, representing various segments of the seafood industry, are expected to participate.

The exhibition

In conjunction with the conference, an exhibition will be organised at the same venue.

The event will showcase and promote products, supplies and equipment from regional and international players.

A total of 20 exhibition booths have been allocated for seafood exporters/importers, processors, equipment suppliers and manufacturers around the Asia-Pacific region and worldwide.

For more information please visit the Shrimp 2019 website:

http://shrimp.infofish.org/

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