

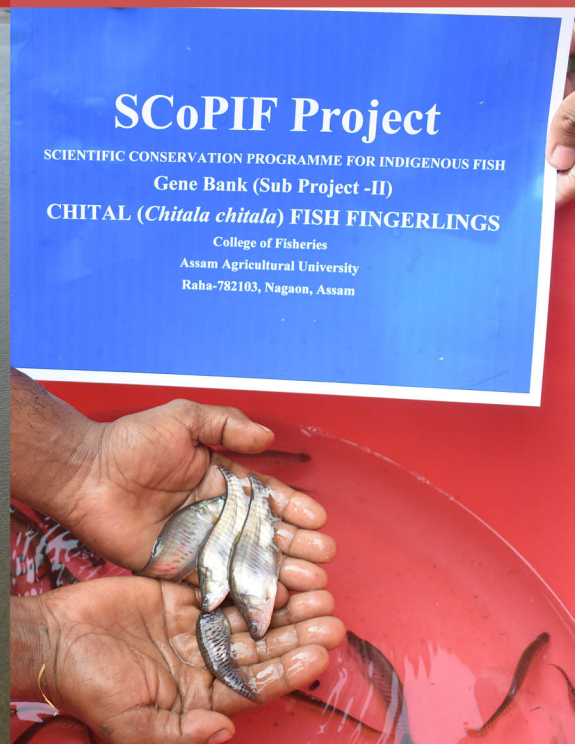
# AQUACULTURE ASIA

Breeding a near-threatened featherback

Wild seed collection and farming of the catfish *Mystus gulio*

Freshwater pearls

Next generation probiotics



## SCoPIF Project

SCIENTIFIC CONSERVATION PROGRAMME FOR INDIGENOUS FISH

Gene Bank (Sub Project -II)

CHITAL (*Chitala chitala*) FISH FINGERLINGS

College of Fisheries

Assam Agricultural University

Raha-782103, Nagaon, Assam



## 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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# AQUACULTURE ASIA

## Ditch the dogma: It's not productive

The human development community is hounded by a fascination with dogma: A principle or set of principles laid down by an authority or an apparent "consensus" as *incontrovertibly true*. Every so often a new dogma will emerge, and people will scramble to adopt the new wisdom and jargon in their project proposals and publications.

Which would be fine, except that usually, the new principles aren't incontrovertibly true. They are usually partly true, perhaps even mostly true, or true in certain circumstances. But in many cases "the truth", as it is presented, is actually a view or paradigm that represents the interests of influential lobby groups.

Complex issues are reduced to simple marketing slogans, partly because these are easier to communicate, and partly because the details may not be fully consistent with the high-level "truth" being advanced. But the reality is that our lives and societies are messy and complicated things, even before considering socio-economic and cultural factors. The devil is in the detail, the detail is essential to understanding the issues, and yet it is being cast aside in favour of sound bite marketing and - worse - sound bite solutions.

But the underlying problem is the dogmatic world view of the development community: Dissenting opinions are not well tolerated. If you don't actively champion and blindly support the latest dogma and its associated jargon, then you are at risk of being labelled a heretic. People are afraid to question the conventional wisdom or even to ask for evidence, because they risk being ridiculed, and certainly will find it more difficult to have their work funded or published.

We need to be able to have open discussion and exchange of ideas or we risk losing our ability to make progress. The nuances and adaptations we should make in our approaches to complex and variable development issues are being lost in an over-simplified 'one size fits all' approach. This leads to poor allocation of resources: Some people and issues that don't need attention attract support anyway because they fit a given profile, while other people or issues that genuinely do need attention go without, because they aren't on the list.

The necessity to tailor interventions to the actual needs of stakeholders is common sense. It is also a recurring theme in development. And yet somehow assessment of actual need is frequently subordinate to the ideologically driven priorities of development agencies, where certain needs are presumed and resources are allocated accordingly.

We have all seen unsuccessful or inappropriate development projects.

In my opinion, better results will be had if people ditch the dogma and stop making assumptions. If you want to know what the real needs are, you need to go and look. If you're turning up with a list of things to fix in your pocket, you're pursuing your own agenda. If you want to know if an intervention was successful, you need to be able to conduct an objective assessment and you need to be open to the possibility of failure.

*Simon Wilkinson*

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# Habitat breeding and seed rearing of a near threatened featherback, *Chitala chitala*

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*Broodstock.*

Globally, the Chinese carps and Indian major carps dominate world freshwater aquaculture production, with about twenty species collectively accounting for around 80 per cent of total freshwater fish production. China, India, Bangladesh and Indonesia account for most of the carp production in Asia at 80%, 12%, 3%, and 1%, respectively.

Low-income people favour carps because of their low price and good taste. In many areas in Asia, carps are the major source of animal protein for the poor. In southeast Asia, carps and other indigenous fish species usually fetch a good market price. Today, due to over exploitation of these species or habitat destruction, the availability of most of the indigenous fish species are diminishing in the wild.

Little work is done on indigenous finfish breeding for diversification of aquaculture in India, with negligible replication of mass seed production technology and grow out culture

practices on a large scale at the farm level. The reason for this is not clear but may be due to a lack of easily replicable artificial breeding technology. Knowledge of artificial breeding is a key aspect as it permits intensive production of a given species under controlled conditions and allows for continuous production of juveniles for restocking natural or artificial water bodies.

Artificial breeding of threatened species for restocking in their natural habitat or to establish gene banks aids conservation through captive breeding programs and has the potential to generate new employment opportunities for rural people. The north-eastern states of India, including Assam, have rich and varied fisheries resources. However, there has been a drastic reduction in the abundance and range of many species due to habitat modification, anthropogenic factors and overexploitation.





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*Adhesive eggs of Chitala chitala.*

To promote the sustainable use of a new candidate species including enhancement of wild stocks and systematic conservation, a good scientific understanding of their biological attributes and culture potential is necessary. Here we present the biological aspects of breeding and larval rearing protocols of the humped featherback *Chitala chitala*, which has been prioritised as a new candidate species for freshwater aquaculture in India.

## ***Chitala chitala* - a new candidate species**

The humped featherback, *C. chitala* is considered to be one of the most commercially important food, sport, aquarium and highly priced cultivable fish. It is known as the 'king' among all cultivable fishes in Assam. It is commonly known as 'featherback' due to the presence of a very long anal fin originating from opposite to mid pectoral and confluent with caudal fin giving the appearance of a feather. Chital is highly prized among the fish-eating population of Assam. The market price of this fish is almost double than that of the Indian major carps and exotic carps. Chital is a highly priced cultivable fish due to its rarity and delicacy having very rich nutritive value.

However, over exploitation, habitat degradation, pollution and related anthropogenic pressure on their natural habitats has considerably reduced the population of this species by 50-60% during the last decades. The Indian biodiversity portal and IUCN Red List has categorised *C. chitala* as a Near Threatened species. Regarding conservation of this species, the first attempt at captive breeding and seed production of this highly priced fish in India was made by U.K Sarkar in 2006. A few attempts have been made by farmers to develop *C. chitala* culture based on wild-caught juveniles and a maximum growth up to 1-2 kg/year has been obtained under polyculture systems. As per the package of practice developed by Assam Agricultural University, *C. chitala* can undergo polyculture with carp species to make aquaculture more remunerative to the fish farmers. Chital can be stocked at an inclusion level of 5% in polyculture pond when carp species attain a weight of 200 -250 g during culture. With the success of the captive breeding and seed production of *C. chitala*, young can be released into the natural waters for rehabilitation and restoration of the species in wild aquatic ecosystems.





Incubation of Chital eggs in Cement cistern.



15 days old larvae of Chital chitala.

### Reproductive Biology of *Chitala chitala*

Age at maturity	3rd year of life
Sexual dimorphism	Females bigger in length than male; abdomen bulged externally in females; males possess pointed genital papillae
Breeding periodicity	Peak time in mid-May to June
Gonadosomatic index	2.8-5% in fully matured fish
Fecundity	10,000-13,000 of egg per kg body weight.
Parental care	Male and female take active part and is very prominent



## Feeding biology

Feeding is one of the most important aspects of fish biology. A sufficient number of reports are available regarding the food and feeding habit of *C. chitala* from India. The fish is carnivorous, with adults feeding on carp fry and aquatic insects. The larvae of chital can be fed on a wide variety of materials including boiled egg yolk, chironomids and tubifex worms. The fingerlings prefer carp spawn and dry feeds comprising formulated feed mixture of 40-50% fish meal along with mustard oil cake and rice polish. The adult fishes weigh 1-2 kg in polyculture ponds and comfortably accept farm made feeds given to the Indian major carps and exotic carps.

## Habitat breeding of *Chitala chitala*

Captive breeding of *C. chitala* was performed in farm ponds of one hectare area at the College of Fisheries, Assam Agricultural University, Raha under the Live Gene Bank Project of Department of Fisheries, Government. of Assam. In ponds, its spawning commences immediately after the early monsoon rains following dilution of pond water to some extent. The peak season for habitat breeding of *C. chitala* has been reported to be in the first quarter of May to June. However, the extended breeding season lasts up to the month of July-August. Captive spawning of chital was observed at the fishponds of College of Fisheries, Raha, where spawning pairs of male and female fishes move together near the artificial spawning grounds. Chital generally spawns on hard substrates such as wooden planks, old car tyres, tree trunks, bamboo poles and so on. At the fish farm of the College of Fisheries, Raha, most of the egg deposition was recorded in old car and bus tyres. A few eggs were laid on the bamboo poles and the wooden trunks of trees. Chital eggs are of 6.0-6.5 mm diameter and are adhesive in nature.

The brood fish were initially procured from Pabhoi Fish Farm in Biswanath District of Assam and were transported to the College of Fisheries. The brooders were raised for one year and were fed daily with a mixture of fish meal, mustard oil cake and rice bran @ 3% of body weight. During the broodstock culture, small carp fingerlings were released into the fish ponds which became an additional source of live food for the brooders. The brooders were not induced by hormone injection, instead natural breeding was performed by providing artificial substrates and fertilised eggs were collected. Natural breeding was triggered by pumping freshwater into the broodstock pond. The maximum number of eggs were collected during the months of May-June, with 500-600 eggs recovered from spawning substrates during the peak breeding season.

## Incubation and hatching

During the months of May-June, the breeding substrates were periodically checked for deposition of fertilised eggs to confirm the ovulatory response of fishes. Swollen eggs of 6.0-6.5 mm diameter were found attached to the substrates with maximum deposition on the tyres of old cars. Specialised wooden boards of size 135 cm × 60 cm × 45 cm were also used as egg collectors in the brood stock ponds but no deposition of eggs was observed on these. The tyres loaded with fertilised eggs were transferred to three cemented tanks containing nylon hapa with continuous water supply

throughout the incubation phase. The unfertilised eggs were opaque, spherical and whitish in colour measuring 2.0-2.5 mm in diameter, while fertilised eggs were yellowish, transparent, spherical and adhesive in nature. The incubation period of eggs in the hatching hapa was 10-15 days during the experiment. The newly hatched fry of 1-3 days tend to attach to hard substrates and possess a bulky yolk sac with a conspicuous network of blood capillaries. Absorption of the yolk sac was completed by most of larvae 12-14 days after hatching. During the breeding season extending from May to July, 1,000-1,200 of chital fingerlings were successfully reared under captive conditions.



# Wild seed collection and modified-extensive farming of *Mystus gulio* in inland water bodies of South 24 Parganas, West Bengal



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70 g *Mystus gulio*.

## *Mystus gulio* in pisciculture

The cultivable small- to medium-sized bagrid catfish *Mystus gulio* is a mangrove fish species, inhabiting estuarine brackishwater wetlands, tidal rivers and streams, creeks and confined freshwaters, and sold as a high-priced food fish in retail markets in cities and towns in southern West Bengal. It is distributed in the lower-most saline zone of the Hooghly estuary, and also encountered in the Sundarbans estuarine complex where it is commonly found at 10-20 cm length<sup>1</sup>, although it spawns in freshwater. Among the farmers of fourteen and six brackishwater Blocks in South and North 24 Parganas districts respectively, where both fresh- and brackish-water habitats exist, *M. gulio* ('nona tengra' or 'bherir tengra' in Bengali vernacular) is being extensively adopted important new species in both freshwater and brackishwater fish culture in village ponds (old backyard semi-managed and well-maintained; also in those having quite high iron content and turbidity) in modified-extensive mixed culture system.

With other brackishwater fishes, *M. gulio* is a good component in culture-based fisheries in canals in the Indian Sundarbans region. It adapts and grows well in freshwater ponds and is recommended for freshwater fish culture in areas of the Sundarbans vulnerable to 5-10+ ppt saline water inundation<sup>2</sup>. A production of 1,200-1,400kg/ha is achievable during April to July and it acts as bottom cleaner, consuming

detritus in polyculture ponds (Dr D. De; excerpt of talk on 'Brackishwater aquaculture opportunities and challenges for meeting livelihood demand in the Indian Sundarbans', 11/1/2020, Kolkata).

In inundated freshwater ponds turned brackish due to natural calamities in the Sundarbans, *Tilapia nilotica* and *M. gulio* can survive. A cluster of villages namely Amjhara, Taldaha, Khorimachan, Phulmalancha, and Sambhunagar-Chunakhali located on the eastern side of the Matla River in Basanti Block in South 24 Parganas are a 'nucleus' of naturally-occurring *M. gulio* seedlings and seeds, exploited every year in good quantity and sold to fish farmers by local seed traders. The Kakdwip Research Centre of ICAR-CIBA in West Bengal developed a comprehensive package for controlled breeding, larval rearing and farming of *M. gulio*. Sri Rabiul Islam Sekh at Namkhana Block in this district developed cost-effective and farmer-friendly modular homestead hatchery technology in 2018<sup>3</sup> and is continuing with support of the institute. Now *M. gulio* farmers in southern West Bengal can avail for its seed, both wild-collected and induced-bred, for stocking in freshwater and brackishwater ponds and larger fish farming areas, viz., rice fields turned freshwater body, ricefield-pond complex, 'mithen gheri' or freshwater ponds of 10,560-26,400 m<sup>2</sup> or even more in area.





A stretch of the Matla River during ebb tide.

Scientists at Ramkrishna Ashram Krishi Vigyan Kendra, Nimpith, South 24 Parganas conducted on-farm trials on increasing profitability from carp polyculture ponds (650 m<sup>2</sup>) by introducing *M. gulo*. Village farmers practice polyculture of major carps in freshwater ponds with proper pond management and application of supplementary feed @ 3% of body weight daily; carp fingerlings stocked @ 10,000/ha and additionally *M. gulo* fry stage stocked @ 5,000/ha and 7,500/ha as two technology options. On farm trials have also been conducted on enhancing profitability by culturing *M. gulo* in small monoculture freshwater ponds of 325 m<sup>2</sup> in the Sundarbans region<sup>4</sup>. Bacterial ulcers, tail/fin rot, and fungal infections are sometimes observed in air-breathing catfishes but not in *M. gulo*. Other merits include its good market demand and rate (even 7.5 cm stage sold @ INR 300/kg in district wholesale markets); it may be cultured with monosex tilapia, *Pangasianodon hypophthalmus*, major carps and other fishes; accepts boiled broken rice, broken maize grains, byproduct of wheat flour 'gomer bhushi', poultry litter mixed with powdered floating feed or wheat flour as feed. The 40-70 g farmed *M. gulo* are sold at much higher price in areas of Basanti and Gosaba near to the Sundarbans when tourists arrive during the year-end.

## Canals in the Sundarbans region

Canals, inundated paddy fields, backyard ponds, large natural water bodies and excavated ponds form five distinct types of freshwater niches in the Sundarbans<sup>5</sup>. The tidal brackishwater Matla River runs in a north-south direction and is a major riverine system in the Sundarbans region. Its tributaries include the Karati, Rampura Khal, Atharobanki and



A canal in Amjhara-Taldaha village.

Bidyadhari rivers. A large number of canals ('gang') in the Sundarbans, 500-2,500 m long are fed by tidal waters from their parent rivers and through connecting channels. Adult *M. gulio* enter canals forming natural populations. During the monsoon, water enters canals when the river water level is high<sup>6</sup>; such inundation canals are fed both by tidal flush and freshwater influx.

## Open fields – repository of *M. gulio* seed

The occurrence of *M. gulio* seedlings observed over the last 20-30 years has occurred exclusively in vast stretches of agricultural land on low-lying extended floodplain ('gher' or 'gheri') in the afore-mentioned villages. With onset of the first pre-monsoon and monsoon rain in March-April and April-May and during the first full moon and new moon periods, large (75-150 g; 12-15 cm) mature *M. gulio* of both sexes leave the Matla River and canals near the river bank as the water rises, and migrate into extended paddy fields and gher (individually 66,000 – 528,000 m<sup>2</sup>) with shallow (7.5-20 cm) stagnant water for spawning. Other fishes such as *Channa* sp, *Anabas testudineus* and *Mystus* sp. also enter the paddy fields to lay eggs in monsoon during paddy sowing season as waters flow in from adjacent beels<sup>7</sup>.

Female *M. gulio* become gravid in early summer with occurrence of mild rainfall. Brooders move into paddy plots from estuarine wetlands, unexploited brackishwater canals (unfished and water never drained) that have connections to the Matla River through sluices, semi-derelict ponds and common village ponds lying near to the fields in the afore-mentioned villages. The sluice gates of canals are often manually opened in that time of year. Smaller-sized brooders (50-75 g) move into the same plots in the next 30-45 days and spawn.

In the Sundarbans, breaching in river bundhs facilitates the inflow of water into canals and entry of *M. gulio* brooders into adjoining fields. After spawning, adhesive fertilised eggs are laid over the submerged portion of young grasses; parents provide oxygenation to developing eggs with their tail. The fecundity of 50-70 g brooder females (6-8 months old) is 5,000-8,000<sup>8</sup>; that of females one year old or more (100-200 g) is higher.

Often, mature *M. gulio* migrate and enter into fields from canals as water flows out from paddy fields. Fish farmers prefer seedlings resulting from the first spawning of the larger brooders, as they have better survival and fast growth when stocked in ponds. According to seed traders, the seedlings of March-April attain 100-150 g by next April or April-May in culture ponds; whereas seed of the next 1-2 months takes 14-18 months to reach this size.



A rice field-pond complex.



## ***M. gulio* seed collection and supply**

From March-April and April-May onwards every year around seven days after the the first rain, 7-10 mm *M. gulio* seedlings or spawn (5-10 days old; cumin seed in size) are exploited by many women belonging to economically-poor local households and traditional fish seed collectors over the next 3-4 months using fine-meshed nylon nets 1.5-2.0 m long and 45-50 cm high, dragged forward along the bottom of fields by two people bowing down. Every pair of women can collect around 50-250 g seedlings working from 7.00 am-1.00 pm and 500-800 g by more impecunious women (fewer in number) who work till 6.00-6.30 pm, eliminating early frog larvae (if any) from their collection. Seedlings of *M. vittaus* ('sona tengra') comprise only 10-15% of it. Seedlings from every *M. gulio* female congregate or remain in a small grassy area, almost sticking onto the grass surface with their head end. Naturally collected early season seedlings are bought by farmers from seed traders of these villages @ INR 2,200-2,500/kg, size: 45,000-55,000/kg (35,000-40,000/kg according to another trader).

Seed collectors exploit advanced fry (6.35-8.90 cm; 300-450/kg) from canals during August and August-September in same year, which are sold to farmers @ INR 700-800/kg. In October, 5.0-6.35 cm seeds also available in canals (600/kg) in addition to larger sizes, captured and sold @ INR 400-600/kg to farmers. In the beginning of the season, *M. gulio* seed traders buy seedlings from collectors @ INR 1,600-2,000/kg; its price falls towards the middle and end of season. A few weeks later, from April-May, the 25-30 days old *M. gulio* seeds (8,000-12,000/kg) are also captured from fields by village women and seed collectors singly using circular nets of 60 cm diameter and sold to traders @ INR 700-900/kg. Traders transport and supply oxygen-packed *M. gulio* seedlings to farmers mainly in Patharpratima, Namkhana, Bishnupur-I, Diamond Harbour-I and II Blocks in South 24 Parganas 30-80 km from home and North 24 Parganas (Sandeshkhali-I and II, Basirhat, Hasnabad Blocks).

Presently 12-14 professional *M. gulio* seed traders in afore-mentioned villages (i.e., in entire West Bengal) and 6-8 persons exclusively in Amjhara, Taldaha, and Khorimachan villages supply the wild-collected *M. gulio* seedlings and seeds to pisciculturists from the entire area, representing



Advanced fry of *M. gulio* captured from canal.



Author right with *M. gulio* farmers at Jharkhali.



Author with *M. gulio* farmers beside the Hatamari River.



Author with three *M. gulio* farmers.



A few pond-grown *M. gulis* of 70-80 g.

some 200 square kilometres. Sri Balai Naskar, age 69 at Taldaha-Shikaripara Village in Basanti has been collecting and trading *M. gulis* seed since 1995 and was the first person to initiate collection and trading of naturally occurring *M. gulis* seedlings/seeds in West Bengal. Younger son Tapas Naskar works with him, while elder son Khokan Naskar is also an established and successful *M. gulis* seed trader. *M. gulis* seedlings and seeds are also available around the same time of year in fields in Canning-II and other places in both districts on river banks and associated canals but no *M. gulis* seed traders exist there and local people aren't interested in collection, so the resource is left unexploited.

Traders collect *M. gulis* seedlings from women and other persons at home in evening and transport them overnight, selling them within 8-30 hours, maintaining the seed on wheat flour in cotton cloth hapa enclosures fitted in homestead ponds for brief period. Prior to beginning of the season, they survey the possibility of sufficient occurrence of *M. gulis* seedlings over the next 3-5 months in specific zones of open fields in afore-mentioned villages and areas of the neighbouring Canning-II, Hasnabad Blocks. Women and *M. gulis* seed collectors are informed accordingly. During August-early October, larger *M. gulis* seeds are collected from deeper areas on the sides of paddy fields; the very small ponds without embankments. For seed traders in these villages, the profit margin from sale of every kilogramme of *M. gulis* seed is INR 200-450 in the beginning of the season and INR 50-80 towards the end. As recruitment of wild *M. gulis* seed is observed in nature by collectors, 10-25% of it (which

is a considerable quantity) could be captured from open fields and nurtured in well-managed ponds; while the remainder survives in the wild to replenish natural stocks.

Sri Naskar opined that in every 640 m<sup>2</sup> polyculture pond, 250 g *M. gulis* seedlings (30,000-35,000/kg) may be stocked in addition to carp fry. In monoculture ponds, 8-10 kg seedlings may be stocked exclusively and propagated, attaining marketable size of around 8.9-10.2 cm (20-50 g) in 90-100 days. Dry wheat flour can be fed daily up to 2.5 cm and subsequently commercial floating fish feed (INR 90-100/kg). Growing *M. gulis* also accepts soaked mustard oil cake and by-product obtained from the Ghatakpukur leather complex near Kolkata where animal skin and cartilage processed and converted into leather ('sukno jhilli'); but this is controversial and so is not used at present. He maintains a stock of *M. gulis* seed (around 7 kg @35,000/kg) stocked in a 96 m<sup>2</sup> pond which reaches 2,000-2,500/kg (22-25 mm) in 35-50 days. These are sold to farmers @ INR 700-900/kg as demand arrives. Wheat flour and/or fine particles of boiled eggs are fed to growing seedlings every day.

At Chunakhali-Mirsahebpara and Gongorpara villages near the Hatamari River, 12-24 mm *M. gulis* seed (2,000-2,500/kg) captured from fields during June-July are sold to freshwater fish farmers in Deuli, Jibontala and other distant villages @ INR 500-1,200/kg, stocked in large freshwater bodies of low depth. Seeds of 10-15 g are caught in traps placed near sluice gates when water is let out from *M. gulis* farming plots into canals during October-November and preparations are





Net for catching 15-25 mm *M. gulio* seed.



*M. gulio* 15-20 g in a sample netting.

made for summer paddy farming. Reputed fish breeder and farmer Mir Amirul Islam stocks 5 kg of *M. gulio* seed (10,000) / 1,320 m<sup>2</sup> in monoculture ponds and 10 kg in his 13,200 m<sup>2</sup> gheri at a low density in the presence of major carps. He uses 1 mm pelleted feed during the initial few days of farming (INR 65/kg) and 3 mm pellets (INR 52/kg) later on. The fish attain 80-100 g when harvested in April-May of the following year. A uniform size is obtained in 8-9 months of culture with very low mortality during winter months if fishes are fed properly, otherwise size variability and slow growth is evident (maximum 40 g). The 80-100 g *M. gulio* are sold @ INR 500/kg in the local fish market but reach INR 650-700/kg in Topsia wholesale market, much nearer to Kolkata city. He often maintains the early stages in large hapa enclosures in ponds for a month up to 36-48 mm and subsequently stocks them in large water bodies; higher survivability is observed.

## Farming of *M. gulio* in freshwater bodies

In a few places in Basanti Block, *M. gulio* (600/kg at stocking) is cultured in large water bodies of 5.28-6.6 ha in association with other finfishes and giant prawn. Almost every household in the Sundarbans region has a freshwater pond, typically around 200-320 m<sup>2</sup>. Some farmers in Nafarganj village, Basanti and other Blocks in South 24 Parganas do *M. gulio* culture in 320-1,320 m<sup>2</sup> ponds with *Heteropneustes fossilis* or *Puntius sarana*. A fish farmer in Jyotishpur Village mentioned that in monoculture in well-managed brackishwater ponds of maximum 105 cm depth, *M. gulio* can attain 100 g in 120-130 days, stocked @ 10,000/bigha (size: 500-1,000/kg) with 70% survivability, fed on commercially pelleted feed with 28-30% protein content. Harvested *M. gulio* are sold @ INR 450-500/kg. In these villages, 2.5 cm seeds captured in fishing traps and nets from naturally-occurring canals and water-logged fields in June-July. Later stages of 10-20 g are captured and sold locally to farmers during September-early November (while post-monsoon rainfall persists) in Notunhat market @ INR 120-140/kg.

Sri Prabhas Jana's pond ('fishery') in Dholar-Kachharipara village is 200 m from the eastern bank of the Bidyadhari River in Basanti but of zero salinity; *M. gulio* is cultured with Indian major carps and 100-150 g (15-20 cm) of specimens



*M. gulio* 15 g at Canning fish market.



Female *M. gulio* 125 g.





*M. gulio* 20 g grown in freshwater pond.

of the latter are harvested when ponds are dewatered during February-March to April-May. *M. gulio* of 15-20 g in September-October attain maturity in next April-May. The 20-40 g stages are sold in local markets @ INR 350-400/kg (more in Kolkata city and sub-urban fish markets) in live condition without water. Dead and ice-preserved *M. gulio* fetches only INR 100-150/kg as the body colour turns whitish and is unappealing to buyers. Some farmers harvest at the end of November as it only feeds a little and the body turns thin in winter with low water temperature. Mature *M. gulio* enter partially-inundated fields in pre-monsoon and monsoon, and move into canals and creeks during post-monsoon flood-like situations. They may remain in deeper areas of paddy fields.

In deeper areas (90-105 cm water depth) on the sides of paddy fields, monsoon paddy saplings are sown and grown in field and *M. gulio* achieves good growth in the former. In early December, large *M. gulio* fry are captured quite easily from canals as the water table recedes. Sri Dipankar Bera at Patharpratima Block has success in polyculture of milkfish *Chanos chanos* and *M. gulio* (bottom feeder) in freshwater ponds; the latter attained 40-60 g from 36 mm stage in eight months. In 1,320 m<sup>2</sup> of such ponds, 20,000 *M. gulio* fry (induced-bred; 24-36 mm) may be stocked and fed a mixture of fish meal 300 g, mustard oil cake 300 g and rice bran 400 g as dough balls in small earthen containers ('maateer maalsa')

@ 8-10% of body weight daily. Market-sized *M. gulio* of 40-50 g are sold locally @ INR 350/kg; the cost of production for 1,000 g fish is estimated to be INR 180 (Sri D. Bera; personal communication). It consumes quite a high amount of pelleted feed quickly in monoculture ponds; the 2,000-3,000/kg stage may be stocked. In monoculture ponds with greenish water, farmers may stock *M. gulio* @ 600-700/40 m<sup>2</sup>, 1,200-2,000/kg size; 7.6 cm *M. gulio* are sold at Rs 350/-/kg in Bangladesh. If 10,000 seed are stocked weighing 10 kg, then 2 kg commercial pelleted feed (0.8-2 mm) should be applied daily in two halves. Broken maize, mustard and soyabean oil cakes can also be used. *M. gulio* seed of 2,800/kg size grow up to 200/kg in size in 16 days. Stocking of grass carp in the same pond and its faecal matter proved useful for *M. gulio* growth. Every 10,000 fish (20-22 g at stocking) require 5-6 kg feed everyday (Courtesy: Bala Fisheries, Jessore, Bangladesh). From 5 g (200/kg) size onwards, pelleted feed applied @ 5-6% of body weight two times daily (Courtesy: Desi fish farming you tube channel)

During high tide in canals in full moon and new moon days beginning in May till July-August ('snarasarir kotal') in different areas in the Sundarbans region, villagers report the occurrence of *M. gulio* seeds (2,200-25,00/kg) in good numbers along with seeds of other brackishwater fishes, which are captured and locally supplied to fish farmers @ INR 200-300/kg who in turn randomly stock about 5-7 kg seeds in every



130-400 m<sup>2</sup> homestead pond where fry/fingerlings of major carps already exist. Little pond management or feeding is practised. Table-size *M. gulio* of 35-210 g, pond-grown using a modified-extensive method, are observed in small rural retail fish markets. In rural ponds of 7,100 m<sup>2</sup> at Joynagar-II Block, *M. gulio* is cultured traditionally in association with major carps (stocking 2,000/10,000 m<sup>2</sup> out of total 15,000/10,000 m<sup>2</sup>) using rice bran and mustard oil cake as feed @ 3% of body weight daily. At stocking, fry/fingerlings of major carps must be larger in size than *M. gulio*.

Sri Debdas Das at Paschim Dighirpar village in Canning-I Block procured and stocked 2 kg of *M. gulio* seedlings (90,000-100,000) from Sri Naskar in a 320-400 m<sup>2</sup> pond with zero salinity, which reached 45-48 mm size (1.0-1.5 g) in 30-35 days of nursery with 80% survival and 8-10 g in 75 days. He stocked *M. gulio* fry in larger freshwater ponds and reared them with major carps (72 mm at stocking; 250-300/kg) and pangas catfish. *M. gulio* attained 50-70 g in 6-7 months and Indian major carps 250-400 g in 5-6 months using home-made wet formulated feed @ 5-8% of body weight daily. If naturally occurring seeds resulting from first spawning of large brooders are first nurtured in small earthen nurseries, and next in grow-out ponds, they exhibit good growth and reach 100 g in 11-12 months, Sri Das opined. He prepares dust-type feed using de-oiled soyabean dust, pulverised groundnut and mustard oil cakes, wheat flour, pulverised and sieved fish meal and semai noodle (rice vermicelli) dust; its cost comes to INR 60/kg whereas commercially-available non-air-breathing catfish feed costs INR 90-120/kg. Feed is applied to *M. gulio* seedlings @ 100% of body weight for the first 7-8 days and reduced to 15-20% on each day of the fourth week. Seedlings are stocked in small earthen ponds of 200-320 m<sup>2</sup> in early May. They prefer to take feed during late evening and night hours and cannot tolerate too much light.

According to giant prawn farmer Sri Ashok Giri at Parbatipur-Birinchibari in Basanti, *M. gulio* grows up to 100-150 g in 8-9 months and is sold @ INR 500-600/kg in Canning town fish wholesale market. He stocked 12 mm *M. gulio* seedlings in mid-June in a 92 m<sup>2</sup> pond, 15,000/kg, total 30,000 after buying @ INR 1,500/kg and 10,000 giant prawn juveniles (4.8 cm). *M. gulio* feeds upon faecal matter of major carps but must be fed properly otherwise considerable difference in body weight occurs at harvest. In polyculture systems, growing *M. gulio* of 7.5-10 cm can hurt carp fingerlings with their sharp dorsal and pectoral spines while feeding, particularly if feed is applied in insufficient amount. It reached 40-50 g in 3.5 months with 80% survival. His *M. gulio* are acclimatised to accept trash shrimp meal (sieved) and/or pulverised fish meal. Both *M. gulio* and prawn accept boiled mussel meat. Prawn juveniles should be of a large size at stocking; otherwise growing *M. gulio* will be aggressive towards them after moulting.

Sri Paritosh Mondal, Sri Haju Jana and a few others at Tridibnagar village in Jharkhali, Basanti are growing *Catla catla*, *Labeo rohita*, *M. gulio* and giant prawns in well-maintained 13,200-15,840 m<sup>2</sup> 'gheri' (perennial freshwater-logged paddy plots 76-120 cm deep, without paddy). Over the last two years, they bought *M. gulio* seedlings at a low price @ INR 700/kg (10,000-15,000/kg; 10-14 mm) in mid-June collected after first rain from fields on Herobhanga river bank at Tridibnagar-Adivasipara. 10 kg of seedlings were stocked in such areas.



*M. gulio* early fry harvested from nursery pond.



*M. gulio* 25-40 g at Sonakhali fish market.



*M. gulio* 30-80 g at Canning fish market.



Small freshwater *M. gulio* farming plots.

The Amjhara-Taldaha-Khorimachan villages are 50-55 km from this extreme end of Basanti. They used small particles of locally-available baked cake and biscuit crumbs (INR 12/kg), wheat flour, mustard oil cake, and non-edible portions of goat from local butcher shops (INR 40/kg) but found these ingredients to be insufficient; *M. gulio* will attain good growth and can be profitably cultured if floating/sinking pelleted feed (INR 1,800-1,900/40 kg; powdered ~2mm diameter) is combined with wheat flour or broken maize (2.5:1) and fed twice a day. *M. gulio* of 40-80 g is harvested at December-end along with paddy when ponds and gheri are dewatered but in conditions of insufficient and improper feeding, the fish attain 15-30 g (5-9 cm) during this period. Local marginal farmers with small land holdings (water-logged paddy fields with 20-25% deeper area as ponds with low embankments or fish refuges, paddy grown in upper region) stocked 2 kg seedlings in every 3,960-6,600 m<sup>2</sup> area but couldn't afford to buy protein-rich pelleted feed. Successful *M. gulio* farmers sold 35-70 g fish @ INR 500-540/kg in Sonakhali wholesale fish market; smaller ones as a second group (50-60/kg) were sold @ INR 350-360/kg.

## ***M. gulio* farming in brackishwaters**

In large brackishwater aquaculture areas in lower the Sundarbans where mullet, pearl spot and tiger shrimp are stocked and propagated, other fishes such as *M. gulio*, *Eleutheronema tetradactylum*, *Scatophagus argus*, *Rhinomugil corsula* and *Terapon jarbua* are observed as potential additional crops because of their natural entry during tidal water exchange<sup>9</sup>. In traditional brackishwater tide-fed impoundments ('bheri'), *M. gulio* attained a final body weight 82.5 g (20.5 cm) from an initial 1.78 g (5.6 cm) in 360 days of rearing, stocked @ 1 piece/m<sup>2</sup>. In brackishwater earthen nurseries, it attained 460 mg (31.15 mm) from an initial 3.33 mg (4.53 mm) in 42 days with 89% survival stocked @ 200 individuals /m<sup>2</sup> <sup>10</sup>. In addition to natural stocking of the 'bheris', shrimp and prawn seed are stocked together with fish seed including Asian seabass, mullet and *M. gulio*, which are wild collected and purchased from local seed traders<sup>11</sup>. But brackishwater fish farmers and 'bheri' owners at Kotrakhali near Hana river in Basanti don't prefer the presence of *M. gulio* in brackishwater bodies and never stock its seed as it preys upon *Penaeus monodon* and *P. semisulcatus* seed. At times when tidal water is let in, larger *M. gulio* escape from bheri(s) through holes previously created in embankments underwater by eel-like fishes. In bheri(s), *M. gulio* feeds largely on algal growths and small crustaceans<sup>12</sup>.





Structure for allowing entry of fish seed into large pond.

At Dakshin Gopalnagar and adjoining villages near the Chinai and Selemari rivers in Patharpratima Block, a brackishwater polyculture 'nona fishery' is practiced by farmers in 3,960-33,000 m<sup>2</sup> impoundments having 10-14 ppt salinity, comprising Asian seabass, mullet, *M. gulio* and *Scatophagus argus*. Fish seed enters plots naturally during rising tides. *M. gulio* grows to 50-70 g in 6-10 months feeding upon plankton and small wild shrimps, is harvested during April-May and sold @ INR 500-600/kg at Kakdwip and Nischindipur wholesale fish markets. If harvested during the previous November-December, it will have a low demand and fetch a low price as familiar marine finfishes dominate the local fish markets at that time of year. According to progressive and innovative brackishwater fish farmer and *M. gulio* seed producer (induced-bred) Rabiul Islam Sekh in South 24 Parganas, the 7-days old larvae (12-14 mm) grow up to 24-36 mm in 21 days in earthen nurseries of 45-60 cm depth when stocked @ 20,000-25,000/120 m<sup>2</sup> with 50-55% survivability. *M. gulio* fry attain 70-125 g in brackishwater grow-out monoculture ponds (7-10 ppt) in six months when stocked @ 20,000/1,320 m<sup>2</sup> with very low mortality. Each *M. gulio* individual consumes 3-4 feed particles (commercial pellets 1mm dia; INR 50-55/kg) per day in each of the first 60 days, 5-6 particles (2 mm diameter) per day from 31-60 days and 10 particles per day from 61-90 days. In Namkhana Block of South 24 Parganas, 5-6 farmers procure *M. gulio* seeds (fry) from Sri Sekh @ INR 2.00-2.50/ piece and are grown in clean brackishwater monoculture ponds of 600-800 m<sup>2</sup> with a hygienic bottom soil, for two

crops per year. Sri Sekh in turn procures and maintains brooder *M. gulio* 100-200 g from them as required. The author learnt from reputed fish farmers Sri Dibakar Majumdar and Sri Debakinandan Patra in Namkhana about their success in grow-out culture of *M. gulio* in polyculture in freshwater and brackishwater ponds.

### End note

Farming of *M. gulio* as practiced by quite a few progressive fish farmers and rural fisherfolks in the Sundarbans region of South 24 Parganas has been brought into focus and discussed in this communication in addition to description of wild seed collection methods. Good-priced farmed *M. gulio* is tastier and nutritious than major carps; remains alive for considerable time outside water; the meat texture of 70-100 g oil-fried *M. gulio* remains stiff and unaltered for 5-6 days at room temperature at home whereas that of major carps starts becoming soft in 1-2 days.

While it is a food fish of the Sundarbans region, nurtured and grown here, demand is increasing in distant fish markets in cities and suburban areas in West Bengal but the quantity supplied presently cannot meet demand. Live *M. gulio* of 50-70 g and above is rarely found in Kolkata city markets.



Sri Sekh whole-heartedly disseminates the technology of seed production to 4-5 progressive fish farmers and gives advice in establishing and operating small-scale hatcheries, so that more seeds are produced and made available to fish farmers in North and South 24 Parganas, Nadia, and Murshidabad districts (as Sri Sekh receives phone calls from freshwater fish farmers on willingness to purchase *M. gulosus* seeds). Ultimately *M. gulosus* culture will flourish and gain wider acceptance. The income from sale of table-size major carps in wholesale markets in West Bengal, typically produced in a 1,320 m<sup>2</sup> pond is about INR 120,000 (considering stocking density of 1,000, selling price INR 120/kg, 1,000 kg production and 1 kg average body weight) but income from sale of *M. gulosus* from the same area is estimated to be INR 425,000 (considering stocking density 20,000, selling price INR 425/kg, 50% survivability, 1,000 kg production and 100 g average body weight); thus income from the latter is about four fold in comparison to the former (Sri R. I. Sekh: personal communication).

According to a study<sup>13</sup>, farmers/fishers in the Indian Sundarbans preferred to culture *Ompok pabda*, *H. fossilis*, *Amblypharyngodon mola* and other small indigenous fishes over major carp species. Fish growers in some villages in the Sundarbans region in coastal West Bengal are doing culture of about eleven indigenous fish species in small backyard ponds, large ponds, drainage canals and low-lying paddy fields<sup>14</sup>. The Sundarbans region harbours both freshwater and brackishwater fish species; *Lates calcarifer*, *Liza tade*,

*L. parsia* and *M. gulosus* are very common in grow-out fish culture in this region<sup>15</sup>. Major carps of 1,000-2,000 g fetch INR 90-120/kg in wholesale markets but farmed *M. gulosus*, *M. vittatus* and *Heteropneustes fossilis* fetch a much higher price as their supply in markets is low in comparison to carps. As catfishes enjoy very good consumer preference and *M. gulosus* is highly adaptable from freshwater to high saline conditions<sup>16</sup>, a high preference for *M. gulosus* farming and production in water bodies with salinities ranging from 0-10 ppt salinities is observed among small- to medium scale farmers in different villages of Basanti and other blocks of South 24 Parganas in West Bengal.

#### References

1. Ghosh, A. (2008). Fishes of Hooghly Estuary. ICAR-CIFRI Bulletin No. 155: 1-42.
2. Trivedi, R. K. (2015). Climate-resilient aquaculture strategies for Indian Sundarbans delta. NICRA-WBUAFS, Kolkata publication: 1-4.
3. Sekh, R. I. (2019). Homestead modular hatchery of brackishwater catfish *Mystus gulosus* for eastern India – an innovative model. In. M. Kumaran, D. Deboral Vimala, M. Muralidhar, C. V. Sairam, K. Sukumaran, P. R. Anand and K. K. Vijayan (Eds.) Innovative approaches and success stories of brackishwater farmers. ICAR-CIBA, Chennai publication: 1-84.
4. Annual Report January-December 2020 of Ramkrishna Ashram Krishi Vigyan Kendra, South 24 Parganas, WB: 1-193.



Pond side at a paddy plot.





*Sluice gate constructed in a canal.*

5. Chatterjee, P. (2010). Utilization of small freshwater bodies and domestic ponds of South 24 Parganas for growing indigenous fish for nutritional security of rural households. Book of Abstract for Golden Jubilee National Seminar on Diversification of Aquaculture using locally available fish species. ICAR-CIFE Kolkata Centre Publication: 140.
6. Gogoi, P., Parida, P. K., Sinha, A., Tayung, T., Suresh, V. R. and Das, B. K. (2020). Canals in Indian Sundarbans - Opportunities towards livelihood improvement through fisheries interventions. *Indian Farming*, 70(11): 10-13.
7. Mondal, B. K. (2013). Dependency on primary economic activity of the Sundarban populace. *The Observer*, 51.
8. Kumar, P. (2018). Low-cost *Mystus gulis* backyard hatchery construction, operation and maintenance. In. Prem Kumar et al (Ed). NFDB sponsored National Training on Seed production and farming technology of brackishwater catfish *Mystus gulis* at Kakdwip RS of ICAR-Central Institute of Brackishwater Aquaculture. CIBA-TM Series 2018, No.11: 25-28.
9. Sundaray, J. K., Biswas, G., Ghoshal, T. K., Panigrahi, A. and De, D. (2010). Species transition and enhanced aquaculture productivity in the lower Sunderbans of West Bengal, India. Book of Abstract for Golden Jubilee National Seminar on Diversification of Aquaculture using locally available fish species. ICAR-CIFE Kolkata Centre Publication: 16.
10. Mondal A. and Mitra A. (2016). Growth, food and feeding habits of *Mystus gulis* in brackishwater traditional impoundments of Sundarbans, India. *Int. Journal of Fisheries and Aq. Studies*, 4(6): 49-58.
11. Arthur, R. I., Bhaumik, U., Pandit, P. K., Saha, S., Garaway, C. J. and Ghosh, T. (2005). Results of management experiments implemented in rice-fish systems in West Bengal as a part of adaptive learning approach. FMSP Project of UK-DFID: 1-99.
12. Pillay, T. V. R. (1956). Land reclamation and fish culture in the deltaic areas of West Bengal. *The Progressive Fish Culturist*, 18 (1): 99-102.
13. Roy, A., Sinha, A., Manna, R. K., Aftabuddin, M. D. and Das, S. K. (2020). Traditional knowledge of the fishermen community of Indian Sundarbans. *Indian J. Fish.*, 67(2): 94-101.
14. Saha, D. (2010). Locally available fish species – a diversified livelihoods option for aquaculturists in coastal Bengal. In. Book of Abstracts, Golden Jubilee National Seminar on Diversification of aquaculture through locally available fish species, ICAR-CIFE Kolkata Centre publication: XV.
15. Sundaray, J. K., Chakrabarti, P. P., Mohapatra, B. C., Das, A., Hussain, A., Ghosh, A. and Hoque, F. (2019). Freshwater aquaculture in Sundarbans India. In. H. S. Sen (Ed.) *The Sundarbans: A Disaster-Prone Ecoregion: Increasing Livelihood Security*, Springer Nature publication: 297-322.
16. Rao, G. R. M. (2002). Catfish culture – time to take off. In *Souv. Sixth Indian Fisheries Forum*, ICAR-CIFE Mumbai publication: 143-147.

# Freshwater pearl culture practices and challenges in India

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As the dictum says 'rare objects have more value'. Human-kind tends to value rare objects such as precious metals and gemstones. Pearl is the only gemstone produced by a biological entity and may be considered an organic gemstone. Molluscs, an ancient group of life, can produce pearls as an immunological response to a foreign particle. Studies have reported that the pearl-producing molluscs first appeared 530 million years ago<sup>1</sup>. However, not all molluscs can produce nacreous pearls, only the bivalves can. About 10,000 species of bivalve mollusc have been reported across the world<sup>2</sup>, but pearls of commercial quality are commonly produced from a few selected bivalve species. India harbours around 3,270 molluscan species including 1,100 bivalves<sup>3</sup> and as many as 625 species of marine bivalves have been reported from India including 88 that are endemic to Indian waters<sup>4</sup>.

In the past, pearls were procured by dissecting mussels collected from nature. This process was cumbersome and it often lead to the extirpation of molluscs from the ecosystem. In 1907, a Japanese scientist Tokichi Nishikawa unravelled the mystery behind the pearl production and proposed the 'Pearl Sac theory'<sup>5</sup>. As per this theory, a pearl is produced by the outer epithelial cells of the mantle tissue (mother of pearl) and can be induced by placing foreign bodies/parasite/a

lesion into the mantle tissues. China pioneered the culture practices of freshwater mussels and produced Buddha image pearls in *Cristaria plicata*<sup>6</sup>.

Mikimoto Kokichi, a Japanese entrepreneur known as the founder of modern cultured pearl, began mass scale pearl production and commercialised the technology. He established the Mikimoto Pearl Company at Ginza, Japan and developed the half round pearl concept and also the mabe pearls. Later, Japan commercialised the culture practice in *Hyriopsis schlegeli* and also standardised the pearl production technique<sup>6</sup>. Since then, the market for pearl has been expanded several fold and now it is a multi-billion dollar sector of the aquaculture industry. In recent years, freshwater pearl culture has accounted for the major portion of the total pearl production. Freshwater pearl culture offers many advantages over its marine counterpart in terms of abundant farming area; absence of fouling, boring and predatory organisms and it is very cost-effective. Several Asian countries such as China, Japan, India, Vietnam, Philippines, Thailand, Bangladesh, South Korea, Malaysia and Myanmar have taken up the culture of freshwater pearl on a large scale and carry out research to meet the global demand for pearls<sup>7,8,9</sup>.



Around fifty two species of freshwater mussels have been reported from India<sup>10</sup>. Among these, those most commonly used species for pearl production are *Lamellidens marginalis*, *Parreysia corrugata* and *Lamellidens corrianus*<sup>11</sup>. These species are distributed in the northeastern, western, central and southern parts of India. The species *Lamellidens* is reported to inhabit stagnant to slow flowing water bodies such as ponds and reservoirs, whereas lotic habitats are inhabited by *P. corrugata*<sup>11</sup>. They are filter feeders and feed predominantly on green algae, followed by diatoms, blue green algae and zooplankton<sup>12</sup>. *L. marginalis* is the most commonly used mussel for pearl production in India rather than *L. corrianus* and *Parreysia corrugata*<sup>11</sup>. In 1987, ICAR-CIFA initiated research on the culture and production of freshwater pearls and since then it has been working towards the development of the technology of producing pearls in freshwater environments. Though the breeding protocol for seed production of *L. marginalis* is under progress, the culture practice for pearl production has already been standardised.

## Pearl farming

### Site selection

The selected site for pearl culture should have a pollution-free water supply and no algal blooms (free of *Microcystis*). Water should be clean with low turbidity as highly turbid water reduces the filtration rate of mussels.

### Obtaining pearl mussel stocks

As mussels take 6-10 years to attain implantable size the mussel stocks for culture/implantation procedures are generally collected from natural water bodies like ponds or rivers. Mussels are hand-picked from the pond bottom and are selected on the basis of their size. Healthy mussels that have attained a length and weight of  $\geq 8$  cm and  $\geq 35$ g, respectively are selected for implantation in the pearl production process.

### Pre-grafting culture

Prior to surgical implantation, the selected mussels are kept crowded for 24-36 hours to ease the relaxation of adductor muscles. The mussels are stocked in FRP/ferro cement tanks in aged tap water at a density of 1 mussel/litre of water. Crowding of the mussels facilitates the smooth opening of the valves for implantation procedure. Pre-operative conditioning is an important pre-requisite to aid surgical implantation due to restricted or no use of narcotising agents in case of *Lamellidens* species, unlike the other pearl forming bivalves. Before starting the surgical procedure, the mussels are kept in an upward facing position for half an hour where the opening part (ventral side) faces upward and the umbo (dorsal side) downwards.

### Grafting

The crucial step in the entire pearl production protocol is the implantation of the nuclei or beads into the mussel. The nucleus, made up of acrylic powder or shell powder can be grafted along with the mantle graft (a sliced mantle tissue taken from the edge of the mantle tissue near the pallial line that is obtained from the mussel that acts as donor mussel) that later develops into a pearl sac. Three different methods of implantation are in practice namely, mantle cavity implanta-

tion, mantle tissue implantation and gonadal implantation<sup>13</sup>. The choice of implantation method depends on the type of pearl targeted i.e. designer pearl, round pearl or rice pearl etc.

- **Mantle cavity implantation:** Out of the three methods, this is the simplest method requiring minimum skill and expertise. In this method, the nucleus is implanted into the cavity between the outer mantle layer and the inner surface of the mussel shell. Mantle grafts are not used in the mantle cavity implantation method as the outer mantle layer serves as the source of nacre secretion.
- **Mantle tissue implantation:** In this method, the nucleus along with the mantle graft is implanted into the pockets made on the posterior side, in both the left and right lobes, of the mantle tissue of the recipient mussel.
- **Gonadal implantation:** Here a small incision is made in the gonad of the recipient mussel and then the nucleus together with the mantle graft is inserted into the incision. A live graft of 2 to 3 mm, taken from the pallial mantle ribbon is inserted along with the round nucleus. Care should be taken to utilise the processed graft within 45 minutes to one hour, otherwise graft will be deteriorated and may not be suitable for implantation.

Implantation can be carried out throughout the year except May-June (summer season) so as to prevent graft and mantle rejection as well as to minimise the post-operative mortality rate<sup>11</sup>. The success rate of pearl production is 60-70% in the mantle cavity and mantle tissue implantation method whereas in gonadal implantations it is 25-30%.

### Post grafting inspection

Following the implantation of nuclei, the mussels are subjected to post-implantation care for a period of 7-10 days to minimise post-implantation mortality and to minimise the rejection of implanted nuclei. During this time, the mussels are treated with broad spectrum antibiotics to minimise rejection and for quick healing of the surgical process. They are kept for 24 hours and after a three day interval again subsequently up to nine days and fed with green algae along with vigorous aeration. Antibiotic @ 1-2 ppm is added to the tanks stocked with implanted mussels immediately after the completion of the surgical procedures and after 24 hours of antibiotic exposure, water exchange is carried out.

### Post-grafting culture (pearl development)

Following post-operative care, the implanted mussels are shifted to the desired culture system wherein they are kept in hanging condition packed in nylon net bags. The implanted mussels are cultured either in FRP/ferro cement tanks or ponds along with other compatible fish species. The culture period of implanted mussels generally varies from 12 to 18 months depending on the method of the implantation followed. Ambient soil and water quality parameters are conducive to the formation of good quality pearl in captive conditions.

### Soil and water quality parameters

The depth of the pond should be 1.5-2.0 m with a clay-soil bottom, slightly alkaline water that is devoid of aquatic macrophytes and algal blooms like *Microcystis* and *Euglena*.

The ponds are employed with bamboo poles as rafts for suspending the implanted pearl mussels. The implanted mussels at a density of 25,000/acre are placed in nylon bags (30 cm x 13 cm; mesh size 1.5 cm) @ 2 mussels per bag and reared. The water quality parameters for freshwater pearl mussel farming should be as indicated below:

Parameter	Range
Transparency of water	40-60 cm
pH of water	7.0- 8.0
Temperature of culture medium	25-30°C
Dissolved oxygen	4-8 ppm
Total hardness	60 ppm
Calcium in water	20-30 ppm
Magnesium in water	5-10 ppm
Total alkalinity	80 ppm
Ammonia	Less than 0.004 ppm

The mussels in pond culture systems are fed with green algae and among them microalgae *Chlorella* is the most preferred food item.

### Culture in tanks

Apart from outdoor culture, implanted mussels can also be stocked and maintained in FRP/ferro-cement tanks till the time of pearl harvest in cases where the farmers have reduced or no access to culture ponds. The mussels can be either placed on the bottom of the tank or suspended in nylon net bags similar as that of the pond culture method. Culturing in FRP/ferro-cement tank also allows the ease of monitoring the condition of the mussels and take the necessary steps in alleviating the difficulties, if any. The ease of removal of the dead mussels from tanks is quite an advantage when compared to the same in a pond culture system, which demands a tedious effort.

### Management measures in tanks

Regular water exchanges, vigorous and constant aeration along with proper feed supplements are the most important pre-requisites for better survivability and production of pearls in tanks. A sudden decrease or increase in the temperature can sometimes prove to be lethal leading to severe mortality of the stocked animals. In such conditions care must be taken to regulate the temperature as per the favourable ambient of 25-30°C, by immediate water exchange, using thermostat to regulate the temperature, strengthening aeration and thinning the stocks.

### Harvest and re-implantation

Harvesting of the pearls is carried out after the designated time period of the culture, based on the implantation method undertaken i.e. 12–18 months. The harvested mussels are sorted based on their quality which is governed by the shape, size, lustre, texture and colour followed by value addition. The mussels after harvest can be reused for implantation only if the mantle tissue method of surgery has been followed. It is necessary that utmost care is taken while obtaining the pearl from the gonads in order to avoid the death of the concerned individuals. In the mantle cavity method of implantation, the mussels have to be sacrificed as the pearl formed attaches to the shell of the animal and requires cutting of the shell to harvest the pearls.



## Challenges in pearl farming in India

Despite its economic value, profitability, minimum labour, there has been relatively little mussel farming in India compared to fish and shellfish culture. Underlying reasons include a low number of freshwater pearl farmers in India and the lack of an organised sector for pearl farming in the country, which is one of the pressing concerns behind the situation. Other factors include a lack of proper brood stock management protocols, scattered availability of mussel brood-stock, non-availability of standardised breeding technology, a lack of standardised water quality management protocols as per different agro-climatic zones of India, few research institutes involved in freshwater pearl mussel farming technology, and a poor extension network to disseminate the existing culture technologies and advances. So, pearl farming, though a profitable business, involves huge challenges which need to be addressed for rapid dissemination of this important technology.

## Future prospects

In order to meet the financial risk associated with pearl farming the Government of India has been providing subsidies and incentives to pearl farmers. Several schemes are being provided by the fisheries department of various states. ICAR-CIFA, Kausalyaganga, Bhubaneswar has been playing a pivotal role in disseminating the freshwater pearl culture technology to farmers, fisheries stakeholders and entrepreneurs who are willing to carry out freshwater pearl



culture. Every year it conducts a training program wherein the candidates receive hands on training on the different methods of implantation and are also trained on the culture practice technologies viz. the pre and post-operative care, food and feeding of mussels, optimal conditions necessary for pearl mussel culture, and water quality management.

## Conclusion

In conclusion, freshwater pearl farming is an emerging sector of the aquaculture commerce in India and laudable progress has been made in developing and standardising the culture techniques. But many obstacles such as the need for proper dissemination of culture technologies among the interested folks, standardised breeding protocol, limited research programmes and so on need to be addressed by the scientific community to boost the contribution of freshwater pearl farming to the national aquaculture produce. Efforts should also be made in producing improved varieties of freshwater pearl producing mussel species and enhancing the quality of pearl produced by the same.

## References

1. [https://rawpearls.com.au/pearl\\_knowledge](https://rawpearls.com.au/pearl_knowledge)
2. <https://ucmp.berkeley.edu/taxa/inverts/mollusca/bivalvia.php>
3. Mohamed, K.S. and Venkatesan, V., 2017. Marine molluscan diversity in India—Exploitation, conservation.
4. Mohamed, K.S. and Sasikumar, G., 2016. Overview of bivalve fisheries of India.
5. [http://eprints.cmfri.org.in/2842/1/Article\\_13.pdf](http://eprints.cmfri.org.in/2842/1/Article_13.pdf)
6. Janaki Ram, K., 1997. Freshwater pearl culture in India. Naga, the ICLARM Quarterly, 20(3-4), pp.12-17.
7. Gervis, M.H. and Sims, N.A. 1992. The biology and culture of pearl oysters (Bivalvia Pteriidae). Vol. 21. WorldFish.
8. Southgate, P.C., Strack, E., Hart, A., Wada, K.T., Monteforte, M., Cariño, M., Langy, S., Lo, C., Acosta-Salmon, H. and Wang, A., 2008. Exploitation and culture of major commercial species. The pearl oyster, 303, p.355.
9. Zhu, C., Southgate, P.C. and Li, T., 2019. Production of pearls. In Goods and services of marine bivalves (pp. 73-93). Springer, Cham.
10. Subba Rao, N.V., 1989. Handbook, freshwater molluscs of India. Zoological Survey of India, Calcutta, pp: 289.
11. Janakiram, K., 2003. Freshwater pearl culture technology development in India. Journal of Applied Aquaculture, 13(3-4), pp.341-349.
12. Mandal, R.N., Kumar, K., Mohanty, U.L. and Meher, P.K., 2007. Estimation of gut contents of freshwater mussel, *Lamellidens marginalis* L. Aquaculture Research, 38(13), pp.1364-1369.
13. Saurabh, S., Mohanty, U.L., Mohanty, J., Jayasankar, P., 2014. Pearl culture technology in freshwater environment. Aquaculture and Fisheries Environment. Discovery Publishing House PVT.LTD, New Delhi, India, pp. 51-78.

# Next Generation Probiotics: Future Therapeutics for Sustainable Aquaculture

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The live micro-organisms that are included in human diet or applied to the aquaculture systems are designated as traditional probiotics. According to FAO, traditional probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host. These live beneficial microorganisms are made use of as functional foods or food supplements. The journey of probiotics started since 1850 when Sir Louis Pasteur discovered lactic acid producing bacteria, the major spoilage organisms of milk followed by its isolation by Dr. Lister. Probiotic research is therefore 168 years old and has led to many findings and applications.

One smarter idea to find an alternative way to overcome side effects of chemical drugs is the use of engineered microbes or designer live microorganisms to produce or deliver therapeutics. This idea became reality through the discovery of next generation probiotics (NGP) or otherwise called live biotherapeutics (LBP) that have been designed to be used as living medicines to treat, cure or diagnose diseases, particularly in humans, that would be impossible with conventional probiotics.

## Next generation probiotics

Currently used probiotic bacteria are drawn from a narrow range of organisms such as *Lactobacillus* spp., *Bifidobacterium* spp., etc. Advances in biotechnological research has lifted traditionally used probiotic bacteria to the next level or "next generation probiotics" (NGP). This primarily refers to those microorganisms which do not have the history of use as probiotics. An enormous amount of research on the microbial consortia (microbiome) of the gut and other body parts has enabled us to find new strains of microbes that do possess some probiotic functions. While cumbersome, this approach has resulted in paradigm shift to next generation level and as a result the probiotic era has moved on from using species-specific probiotics to strain-specific ones. NGP utilise the health promoting capabilities of specific bacterial strains.

NGP differ from traditional probiotics in that they are likely to be delivered under a drug regulatory framework. At present, conventional probiotics are used as either food ingredients or as supplements whereas NGPs are mainly used to treat or cure disease conditions in the body. Therefore, NGPs are considered as therapeutics (drugs) rather than functional foods or supplements, and hence must be delivered under regulations used for registering pharmaceuticals.

The US Food and Drug Administration (USFDA), defines live biotherapeutics (LBP) as biological products that contains live organisms, such as bacteria, and that are applicable to the prevention, treatment or cure of human diseases and are not vaccines. NGPs are generally considered to be LBPs except

there are some operational differences, such as the former are usually identified and characterised by ongoing probiotic research laboratories based upon their microbiome research data, whereas the latter are developed as pharmaceuticals by business-oriented biotechnology start-up companies. Since NGP fall under the category of LBPs or drugs, any probiotic strain that will be used as NGP is subject to the usual pharmaceutical clinical trials and research on its pharmacokinetics, pharmacodynamics, safety and delivery routes. This process is stringent and poses significant hurdles on the pathway of NGP development and commercialisation.

To improve, the efficiency of organisms to be used as NGPs, certain scientific interventions, say to exclude toxic protein production or to improve their probiotic functions, are being made. This brings them into the category of genetically modified organisms (GMO). In the present scenario, the NGP industry is dominated by genetically modified bacteria designed to perform certain desired functions. As GMOs are a controversial topic these interventions in the probiotic sector may result in a reduced consumer preference. The scientific community is recommending that all NGPs should be included under the title of LBPs (drugs) so that consumer acceptance of conventional probiotics can be sustained.

## Current candidate microbes for next generation probiotics

NGPs are more strain-specific than species-specific and it is important to know their health promoting features as probiotics. These features are related to their evolutionary history as in the case of traditional probiotics. For example, *Bacteroides fragilis* produces a toxic protein named fragilysin which is known to be a risk factor for human colorectal cancer. But a particular strain of *B. fragilis* strain ZY-312 is known to have immunomodulatory effect especially the phagocyte promoting activity, thus it can be considered a NGP.

Certain bacteria that doesn't have any probiotic function can also be utilised as live delivery vehicles for bioactive molecules or drugs. These are termed alternative NGPs. For example, *Lactococcus lactis* is normally not a probiotic bacteria but they are commensal to human gut. Researchers proved that they can be engineered to deliver some molecules that modulate inflammation such serine protease inhibitor, Interleukin-10 etc.

Some of the currently considered candidate strains for next generation probiotics are as follows:



**Table 1. Characterised NGP/LBP candidates with their corresponding beneficial effect.**

NGP Candidate	Effect on body
<i>Bacteroides dorei</i> D8	Convert cholesterol to coprostanol in vitro
<i>Bacteroides acidifaciens</i>	Increase IgA
<i>Bacteroides ovatus</i>	Increased levels of anti-TF $\alpha$ IgM and IgG antibodies.
<i>Faecalibacterium prausnitzii</i>	Induction of anti-inflammatory cytokines or reduction of pro-inflammatory cytokines

## Development of NGP/LBPs

It's not easy to identify and introduce an NGP/LBP commercially. The development pathway is rigid and completely covered under the regulatory framework. According to USFDA regulations, a LBP should describe its complete characteristic features before it is applied. They are described as follows:

- Should describe the drug substance including the biological name and microbial strain details. The original source of microbe from which it derived inclusive of its culture history.
- Description about health status of clinical donor and a report of the phenotypic and genotypic expression of the bacterial strain. If we have done any genomic modification to the natural bacteria, a summary must be provided.
- Complete microbiological, biochemical and diagnostic characterisation of the strain including details about the antibiotic resistance.
- A manufacturer must be provided with complete and comprehensive data on mass scaling up manufacturing protocol (good manufacturing practices), infrastructure required and details of any other products produced in the same facility.

The major production challenges faced by the manufacturing sector includes the maintenance of anaerobic conditions in the whole production chain in order to maintain the viability and functionality of bacterial strains during the storage period. Since all identified NGP candidates are completely unable to grow in the presence of atmospheric oxygen (strict anaerobes) compared to microaerophilic traditional probiotics such as *Lactobacillus* spp. and *Bifidobacterium* spp., it is necessary to exclude the oxygen from the whole production line which is more technically difficult.

## Pathway to produce a NGP/LBP

The commercial pathway to produce NGP/LBP and to obtain approval and it's commercialisation from the authority include identification of the NGP/LBP candidate bacterial strain, its complete characterisation, production and scaling-up of LBP and finally the clinical phase trials. Among all these steps, the most crucial and challenging task is the identification of suitable LBP candidates. Most of the time, researchers identify suitable strains based on some hypothetical

approaches such as selecting an organism whose abundance level was depleted during alteration of the microbiome (dysbiosis of microbiome) in connection with any disease condition or on the basis of some organisms having an influence on a particular host pathway or phenotype relevant to a particular disease etc. An alternative is to screen blank unknown strains for a desired in vitro or in vivo probiotic activity.

Once a bacterial strain has been identified, the next stage is to characterise the LBP. This phase may include culture-dependent and culture-independent evaluations such as genome sequencing and screening for antibiotic resistance genes, toxic and virulent genes, enzymatic assays and so on. This step is also highlighted with the safety information regarding the strain, for that cell, animal and ex vivo model trials have to be conducted and documented.

The production phase encompasses the pilot and mass scaling-up of manufacturing protocols, establishment of defined media for the microbial culture, good manufacturing practices and formulation of effective delivery of the LBP stating its survival inside the host and bioavailability. The product approval will be commencing at the end of this phase and continues to the next phase. The usual pharmaceutical trials have to be conducted for commercial licensing and application. This includes three sub-phases: Phase 1 is a first in-human trial for confirming safety and dosage ranges and phase 2 and 3 are conducted on medium and large human populations respectively to know the efficacy, side effects and expanded safety in humans.

Unfortunately, the major challenges and issues faced by the last stage of the pathway is the approval and marketing. The authorisation of any micro-organism as a drug needs to be approved by the registered medical agency or council such as European Medical Agency, European Food Safety Authority (EFSA) in Europe and United States Food and Drug Administration (USFDA) in the United States.

## Scope and applications of NGP/LBP

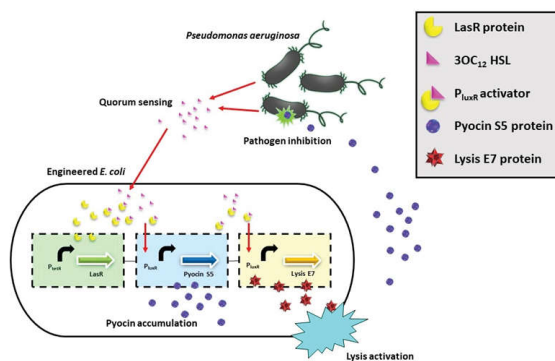
NGP/LBP has a plethora of identified and proven applications which signifies the new genera of probiotics. At present, in every field of science, the suitability of NGP/LBPs has been presented and continues to be evaluated. Some of the recognised fields include drug administration, vaccine delivery, immunomodulation (boosting the immune system), psychobiotics (for the treatment of mental disorders), cancer treatment and prevention, stress tolerance, production of antimicrobial compounds, to understand the pathogenesis of enteric infections.

The scope of NGP/LBPs lies in how we are designing probiotics by genetically engineering bacteria. Researchers are now applying synthetic biological tools and techniques to engineer NGP strains to address specific problems in human medicine and pharmacology. Some of those achievements are illustrated below.

# 1. Engineered therapeutics

Research findings were published in 2011 by Dr. Saeidi and his team from Nanyang Technological University, Singapore. They engineered probiotic *E. coli* (three additional genes were incorporated to the natural genome) to put the quorum sensing ability from *Pseudomonas aeruginosa*, a pathogen causing urinary tract infections and pneumonia in humans and connected it into the pyocin (a toxic protein produced by the *P. aeruginosa*) production. The mechanism they employed is that during infection, the *P. aeruginosa* will form a biofilm over the urinary tract (forms the quorum) and produces quorum sensing molecules to communicate between the bacteria. These molecules can be detected by the engineered *E. coli* (by production of LasR protein). Upon detection, *E. coli* will initiate the production of pyocin protein which accumulates inside the cell. With the help of another lysis protein, the cell wall is lysed allowing release of pyocin molecules. This inhibits the biofilm formation of *P. aeruginosa* (pyocin in high concentration is toxic to *P. aeruginosa* itself) (Illustration 1).

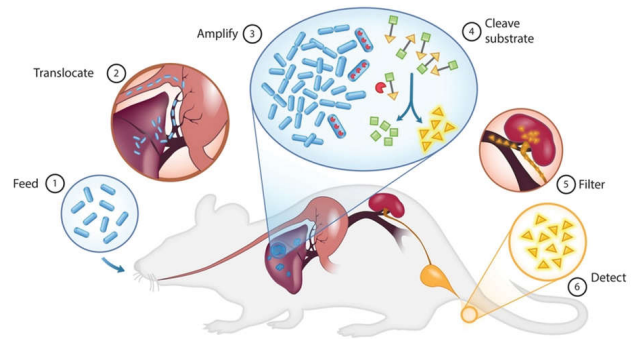
Illustration 1. After Saeidi et al., 2011



# 2. Living medical test

Apart from the duty of prevention and cure, another job that can be assigned to these designer probiotics is to detect specific diseases in body. An engineered probiotic can sense some disease-specific molecules from our body and give us the indication about the disease progression. One such living medical test was demonstrated by Danino et al. in 2015 to diagnose the early progression of liver cancer. They genetically modified a probiotic *E. coli* Nissle (ECN) strain by incorporating LacZ gene into the genome. The LacZ protein has the capacity to cut its respective substrate molecule to two parts. When engineered ECN was fed to mice (pre-clinical model), ECN was localised on progressive tumor tissues. LucGal (luciferin-galactose) substrate was injected into mice through a tail vein. Bacteria-produced LacZ protein specifically cut the LucGal substrate to luciferin and galactose. The by-product luciferin is filtered out through the urine which can be detected using a commercial luciferin-luciferase luminescent detection kit. Even 1 microliter of urine can be used to diagnose liver cancer via this living medical test (Illustration 2).

Illustration 2. From Danino et al., 2015



# Scope of NGP/LBPs in aquaculture

Sustainability is a word we constantly use to describe aquaculture goals. Aquaculture farms often provide suitable conditions for diseases to flourish easily. The use and abuse of chemicals and antibiotics has been a problem in the aquaculture industry, raising concerns about food safety and development of anti-microbial resistant bacterial strains. Probiotics have proved their beneficial effects in disease management, stress control as well as in growth. If traditional probiotics are used as food supplements and as preventive measures for improving health, NGP/LBPs have the scope to treat or cure disease if introduced as an alternative to chemical drugs or antibiotics. Development of effective NGP/LBPs will be a promising potential step to sustainable health management in aquaculture.

Applications such as immunomodulation, stress tolerance, drug and vaccine delivery are fields where we can utilise the potential of NGP/LBPs in aquaculture. Increasing fish microbiome profiling and associated research can help to identify and characterise novel fish specific NGP strains. Interestingly, researchers have started to work on NGPs in the aquaculture sector by improving existing probiotic strains. The application of NGP/LBPs in aquaculture has not yet proceeded far. This may be due to the fact that LBP research is still in its infancy and may still be considered a bit controversial.

# Conclusion

Next generation probiotics (NGP) or live biotherapeutics have opened up a wide array of possibilities of using live microbes as therapeutics and we can refer to them as therapeutic probiotics. They are a promising and eco-friendly alternative to high-impact chemical drugs and antibiotics, highlighting the fact that our own commensal bacteria can act as life-saving medicines to combat deadly diseases. There are some concerns regarding the NGP/LBP as they can involve genetically modified organisms. So future research should be directed towards the problems such as biocontainment of engineered probiotics and interactions between synthetic bacteria and commensal organisms in the gut. Data should also be generated on the aspects of interactions with the host's metabolic and signaling pathways and interactions with the aquatic environment in the case of aquaculture. We need to consider more than just the direct effects of these microbes on the system or host. Of course, we are still at a stage where more questions arise than answers regarding the next generation probiotics.



## References

- Bäckhed, F., Manchester, J.K., Semenkovich, C.F. and Gordon, J.I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc. Natl. Acad. Sci.*, 104(3):979-984.
- Cani, P.D. and Van Hul, M. (2015). Novel opportunities for next-generation probiotics targeting metabolic syndrome. *Curr. Opin. Biotechnol.*, 32:21-27.
- Danino, T., Prindle, A., Kwong, G.A., Skalak, M., Li, H., Allen, K., Hasty, J. and Bhatia, S.N. (2015). Programmable probiotics for detection of cancer in urine. *Sci. Transl. Med.*, 7(289):289ra84-289ra84.
- O'Toole, P.W., Marchesi, J.R. and Hill, C. (2017). Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nat. Microbiol.*, 2:17057.
- Saeidi, N., Wong, C.K., Lo, T.M., Nguyen, H.X., Ling, H., Leong, S.S.J., Poh, C.L. and Chang, M.W. (2011). Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen. *Mol. Syst. Biol.*, 7(1):521.
- Singh, B., Mal, G., Bissi, L. and Marotta, F. (2016). The Holy Grail of designer probiotics: the probiotics with multiple health benefits. *JGDD*, 6:2.
- Sola-Oladokun, B., Culligan, E.P. and Sleator, R.D. (2017). Engineered Probiotics: Applications and Biological Containment. *Annu. Rev. Food Sci. Technol.*, 8:353-370.
- Taylor, C.P. and Lamont, J.T. (2005). Genetically engineered probiotics: a new twist on an old remedy. *Gastroenterology*, 128(5):1509-1512.



## Global Conference on Aquaculture Millennium +20



Feeding an expected global population of 9 billion by 2050 is a daunting challenge that is engaging hundreds of millions of farmers, food processors, traders, researchers, technical experts, and leaders the world over. Fish and other aquatic products from aquaculture can and will play a major role in meeting the dietary demands of all people, while also meeting the food security needs of the poorest.

Farmed fish and plants have long contributed to healthy diets, poverty alleviation and rural development. It is only recently, however, that aquaculture has grown to be the leading source of aquatic food.

To realise the maximum contribution of the aquaculture sector toward achieving the targets set by the Sustainable Development Goals and Agenda 2030, coordinated and accelerated actions are required. Not only must these actions increase sustainable production, but also address the broader value chain, markets, and decent employment.

To accelerate growth and ensure the sustainability of future aquaculture, FAO, NACA and the Chinese Ministry of Agriculture and Rural Affairs invited governments, business, academia and civil society together for the Global Conference on Aquaculture Millennium +20 (GCA +20) under the theme “Aquaculture for Food and Sustainable Development”, to discuss the policy and technology innovations, investment opportunities and fruitful areas of cooperation.

The event was the fourth in a series of development-oriented conferences that began with the FAO Technical Conference on Aquaculture (Kyoto, 1976), the Global Conference on Aquaculture in the 3rd Millennium (Bangkok, 2000), and the Global Conference on Aquaculture 2010 (Phuket).

The goals of the conference were to:

- Review status, trends, and emerging issues in aquaculture development.
- Identify opportunities and challenges in aquaculture and its contributions to sustainable development.
- Evaluate the progress of aquaculture development considering previously recommended strategies and policies at regional and global level.
- Build consensus on priorities and actions needed for advancing aquaculture as a global, sustainable, and competitive food production sector.

To provide context for the discussions, an extensive series of review papers were prepared by teams of recognised experts. These included a series of regional reviews and a global synthesis of the status of aquaculture; and a series of thematic reviews addressing aquaculture systems, innovation, aquaculture’s contribution to the Sustainable Development Goals, feed and feeding, genetic resources and seed supply, biosecurity, governance, social and human dimensions of aquaculture, and value chains and market access.



The review papers, which were open for public comment, are available for download from the conference website at:

- <https://aquaculture2020.org/regional/>
- <https://aquaculture2020.org/thematic/>

Despite delays due to the pandemic, the GCA +20 was successfully held as a hybrid event from 22-25 September, with physical participation at the venue in Shanghai, China, and international participation via video conference.

The conference kicked off on the 22nd with a Workshop on Sustainable Development Goal-aligned *Artemia* Aquaculture (refer separate article, this page).

A total of 1,728 people participated in the event, of which 500 were physically present in Shanghai. Around 45% of participants were academics, 30% were from civil society groups and NGOs, 15% were from the private sector and 10% public sector. Across all participants, 41% identified as female and 52% as youth.

## The Shanghai Declaration

Her Royal Highness Princess Maha Chakri Sirindhorn graced the final session of the conference and the formal adoption of the Shanghai Declaration, expressing her support for aquaculture as a solution to achieve Sustainable Development Goal #2, Zero Hunger.

A key output from the GCA +20, the Shanghai Declaration is a call to action that highlights the principles and strategic pathways to maximise the contribution of sustainable aquaculture in achieving the Sustainable Development Goals, with a special focus on “Leaving no one behind”. The Declaration will guide all players in the development of the industry and towards optimisation of the sector’s contribution to food security and livelihoods in line with the UN’s 2030 Agenda for Sustainable Development.

The Declaration recognises the capacity of aquaculture for further growth, while stressing the need to prevent that growth from impacting ecosystems and biodiversity, animal health and welfare, and social inequalities.

At the time of writing, 42 organisations have submitted written pledges expressing their support for the Shanghai Declaration and their commitment to addressing issues it contains, including universities, research institutes, international organisations, civil society organisations, industry associations and private sector companies.

The Shanghai Declaration and pledges of support are available for download from the conference website at:

- <https://aquaculture2020.org/declaration/>

NACA would like to thank all who contributed to the GCA +20 as participants, authors, speakers or in organising the event, our partners at FAO, the Ministry of Agriculture and Rural Affairs PRC, Shanghai Ocean University and the legion of administrators, translators, editors and others that made it possible.

## Workshop on SDG-aligned *Artemia* aquaculture



With the expansion of hatchery production, the demand for *Artemia* cysts has continued to increase. Annual consumption is now estimated at 3,500 – 4,000 tonnes, which underpins the production of over 900 billion crustacean post larvae and fish fry. The hatchery industry is now valued at more than US\$ 2 billion and is responsible for the final production of over 10 million tonnes of high-value aquaculture species. With approximately 90 percent of the current *Artemia* production harvested from inland salt lakes, the future of the hatchery industry could be at risk and requires urgent attention.

A new international interdisciplinary approach is needed to tackle these *Artemia* issues and opportunities, like the breakthrough in *Artemia* use in aquaculture following the 1976 FAO Kyoto conference.

A workshop on “Sustainable Development Goals-aligned *Artemia* aquaculture” was held simultaneously in Shanghai and online via Zoom on 22 September. The workshop was the first event of the Global Conference on Aquaculture Millennium +20, and was attended by around 400 people from around the world. The workshop was moderated by Professor Liying Sui of the Asian Regional Artemia Reference Center (AR-ARC) and Dr Rodrigo Roubach, FAO. It was organised by FAO, AR-ARC, NACA, the Artemia Association of China and the Laboratory of Aquaculture and Artemia Reference Center, University of Ghent. Min Jiang, Shanghai Ocean University, and Qingyin Wang, Chinese Fisheries Society, China, gave welcome remarks.

The purpose of the workshop was to explore needs and opportunities for a new international initiative to guarantee a more sustainable provision of *Artemia*, both from natural sources and from controlled extractive *Artemia* farming integrated with salt production and other fish/crustacean aquaculture.

The workshop began with a presentation by Professor Patrick Sorgeloos (Artemia Reference Center) providing a brief history of the use of *Artemia* in aquaculture. This was followed by reports on an International workshop on *Artemia* pond production (Meezanur Rahman, WorldFish) and Webinar on the status of the use of *Artemia* cysts in fish and crustacean hatcheries around the world (Simon Wilkinson, NACA).

Tomas Bosteels (Great Salt Lake Brine Shrimp Cooperative) presented on “Sustainable harvesting of natural *Artemia* resources: The Great Salt Lake (Utah, USA) as model case”, and Honzalo Gajardo (Los Lagos University) gave a presentation on “*Artemia* species and strains diversity: threats and potential”. The final presentation was given by Gilbert Van Stappen (Ghent University) “Availability of *Artemia* genome: R&D opportunities”.

Video recordings of the presentations will be published on NACA’s Youtube channel in the near future and will be announced on the NACA website.

## Professor Sena De Silva Memorial Oration, 8 October 2021

Join us on 8 October 2021 for the Professor Sena De Silva Memorial Oration. The topic for the oration is “Aquaculture and marine resources exploitation: reframing the issues”, presented by Professor Giovanni Turchini of Deakin University and organised by the Sri Lanka Association for Fisheries and Aquatic Resources. The oration will be held at 05:00 GMT (15:00 AEST) via Zoom. To participate, please use the following link:

<https://bit.ly/3A2m3ve>



Sri Lanka Association for Fisheries and Aquatic Resources  
SLAFAR





Professor Sena De Silva  
Memorial Oration - 2021

“Aquaculture and marine resources exploitation: reframing the issues”

Date: 8<sup>TH</sup> October 2021 | 10.30 (IST) | 15:00 (AEST) | 05.00 (GMT)



<https://bit.ly/3A2m3ve>



**Professor Giovanni Turchini**  
Professor of Nutrition and Food Science, and Associate Dean Research,  
Faculty of Science, Engineering & Built Environment,  
Melbourne Burwood Campus  
Deakin University, Victoria, Australia

Organized by: Sri Lanka Association for Fisheries and Aquatic Resources (SLAFAR)

## Apply now: Training Course on Mariculture Technology in Asia-Pacific

A free training course on mariculture technologies will be offered online via Zoom from 18 October to 5 November, by the Yellow Sea Fisheries Research Institute (YSFRI), People’s Republic of China. The course will cover:

- Genetics and breeding of mariculture species.
- Large-scale propagation.
- Disease control and prevention.
- Nutrition research and feed development.
- Technology for different farming models.
- Equipment research, engineering and construction of farming facilities.
- Quality and safety inspection technology for aquatic products.

The course is aimed towards officials, researchers and technicians from fisheries and aquaculture departments, research institutions, and enterprises. Priority will be given to personnel from developing countries.

The course is hosted by the Yellow Sea Fisheries Research Institute (“Belt and Road” Training Base for Mariculture Technologies, Ministry of Agriculture and Rural Affairs, PRC) of the Chinese Academy of Fishery Sciences, and NACA. It is organised by the Department of International Cooperation, Ministry of Science and technology, PRC.

### Applications

To apply for the course, please:

- Download the prospectus (refer to the section “Qualifications of Trainees”):  
<https://enaca.org/enclosure.php?id=1174>
- Fill in the online application form at:  
<https://forms.office.com/r/B7A9D1KrRJ>
- Email a recent passport photo of yourself to:  
[ice@ysfri.ac.cn](mailto:ice@ysfri.ac.cn).

**Applications close 11 October** and space is limited, so be quick! YSFRI will advise applicants of their admission status in due course. For more information, please download the prospectus.



# New project on “Blue transformation in aquaculture”

NACA and FAO have signed an agreement to implement a project in support of FAO's Blue Transformation Initiative. The project will identify and document priority thematic areas and innovations contributing to the transformation of aquaculture in participating countries and convene a virtual meeting on country strategies for upscaling innovations, as a lead in to scale up through field projects and capacity building activities in the region.

Aquatic food is an important food resource for human consumption, both for high-quality protein and fat, supplementary and necessary vitamins, minerals and micronutrients. Moreover, the waters that cover most of the global surface offer enormous potential for aquatic food production. The recognition that aquatic foods should contribute a more significant and sustainable share in human food systems is the rationale of the blue transformation initiative of FAO. This initiative recognises the unique benefits of aquatic foods for food and nutrition security, livelihoods, trade, and as the source of the social values that gel many societies together.

As a new concept, blue transformation acknowledges successes while facing the sector's sustainability challenges head-on. To better understand the possibilities the aquaculture sector offers to the blue transformation initiative, it is important to document innovations that will support the sustainable intensification of the sector.

If the current population growth continues unchanged, by 2030 the planet will have to feed as many as an additional 1.5 billion people, 90 percent of whom will live in developing countries and world food production will need to increase 60-70 percent to feed an additional nine billion people by 2050 (FAO, 2019). With land and water finite, feeding the world will require enhanced agricultural productivity and efficiency to produce more food using less resource inputs through production systems that not only conserve, but that actually enhance natural resources. This will require the sustainable intensification of agriculture and food production systems including aquaculture, the use of an ecosystem approach, and a strategy for the integrated management of land, water and living resources that promotes conservation and sustainable use in an equitable manner.

Blue transformation is FAO's vision of how to achieve a balance between the need for positive societal outcomes and ecological sustainability in food production from the blue economy. It has three core components, namely:

- Sustaining fish supplies and feed the world through aquaculture intensification. As aquaculture will need to provide the majority of supply increases – scale-up, address resource-use bottlenecks and transfer knowledge through sustainable development of aquaculture.
- Transforming fisheries through better management. By addressing overfishing and overcapacity, combating illegal, unreported and unregulated (IUU) fishing and rebuilding overexploited stocks, fisheries can improve livelihoods and supply. However effective management is non-negotiable.

- Upgrading fish value chains. Improving the efficiency, viability and inclusiveness of fish value chains. Providing additional supply sources and ensuring socio-economic benefits, just distribution and access.

A video of the concept of blue transformation in aquaculture presented by Dr Simon Funge-Smith, FAO RAP, is available on the NACA Youtube channel at:

- <https://www.youtube.com/watch?v=O8D7If-E4P0>

As the first activity under the project, a virtual consultation was held on 15 September to:

- Introduce the blue transformation of aquaculture.
- Gather input from representatives of NACA Member Governments and Regional Lead Centres.
- Present the current and planned priority areas and innovations identified by the countries that will contribute to the blue transformation of aquaculture.
- Summarise the regional priority areas and sub-areas and innovations contributing to blue transformation of aquaculture in Asia and the Pacific.
- Present the objectives and work plan for detailed country reports on priority areas, innovations, and scaling-up strategies to support blue transformation of aquaculture in Asia and the Pacific region.

Issues raised were many and various, but mainly could be categorised within the blue transformation priorities, which are:

- Governance, sector and policy reform.
- Socio-economic benefits and considerations.
- Biosecurity and disease.
- Feed ingredient and feed technology innovations.
- Genetic improvement, breeding and diversification.
- Digital technologies and intelligent systems.
- Environmental control and regulation.
- Value chain efficiency.
- Climate change.

The next phase of the project will involve preparation of national reports and a consultation on country strategies for up-scaling, to be completed in 2022.

# Webinar on Status of Artemia cyst use in fish and crustacean hatcheries



A free webinar on the Status of the use of *Artemia* cysts in fish and crustacean hatcheries around the world was held on 2 September via video conference.

The webinar is facilitated by the International Artemia Aquaculture Consortium (under formation) as a follow up to the recommendations of the recent article Past, present and future scenarios for SDG-aligned brine shrimp *Artemia* aquaculture in FAO Aquaculture News (<http://www.fao.org/documents/card/en/c/cb4850en/>).

The goal of the webinar was to document differences in practices used by fish and crustacean hatcheries in the use of *Artemia* cysts for the preparation of live feeds. Over time, the practices used by hatcheries in Asia, Europe and Latin America have diverged from the good aquaculture practices for *Artemia* production recommended by FAO in the 1991 Live Food Manual.

Speakers included technical experts using *Artemia* in the production of shrimp, freshwater prawn, mud and mitten crabs, seabass, seabream and other marine fish from Bangladesh, Brazil, China, Ecuador, Greece, India, Spain, and Thailand.

The programme began with a presentation by Patrick Sorgeloos (Artemia Reference Center, Belgium) "An introduction to the *Artemia* cyst hatching process and the crucial parameters to ensure optimal hatching and preparation of *Artemia* for use in the hatchery feeding of fish and crustaceans.

This was followed by short presentations of how *Artemia* cyst decapsulation, cyst hatching, umbrella/nauplii separa-

tion, cold storage, heat-killing/freezing, enrichment, etc. are performed in small- and large-scale hatcheries in the main regions of the world:

- Thailand (shrimp and Asian seabass): Montakan Tamtin (Department of Fisheries)
- Vietnam (shrimp, prawn and mud crab): Nguyen Van Hoa (Can Tho University) and Trinh Trung Phi (Viet-Uc company)
- India (shrimp): Nageswara Rao (All India Shrimp Hatcheries Association, AISHA)
- Bangladesh (shrimp and prawn): Meezanur Rahman (Artemia4Bangladesh EU project, WorldFish)
- China (marine fish, shrimp, prawn and mitten crab): Liying Sui (Asia Regional Artemia Reference Center, AR-ARC) and Song Gao (China Artemia Association, CAA)
- Greece (European seabass and seabream): Dimitris Dimopoulos (Tapias Hatchery of Phylofish company)
- Spain (European seabass and seabream): Gustavo Espelleta (Avramar company)
- Ecuador (shrimp): Stanislaus Sonnenholzner (Centro Nacional de Acuicultura e Investigaciones Marinas CENAIM)



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- Brazil (shrimp): Marcos Camara (Federal University of Rio Grande do Norte) and Cristine Macedo (on behalf of Camar company and Aquatec company)

The last session was a Q&A session moderated by Simon Wilkinson (NACA).

The webinar produced a report with specific recommendations for a follow up meeting on Sustainable Development Goal-aligned *Artemia* aquaculture, held in conjunction with the Global Conference on Aquaculture (refer separate articles, this issue). The workshop contributed to preparation of updated recommendations on how to better use *Artemia* in hatcheries as an important input for a new FAO *Artemia* manual and future training programmes for local hatcheries.

The technical presentations from the workshop are available for viewing on NACA's new Youtube channel at the link below. Please subscribe!

<https://bit.ly/artemia-2021>