Research and Innovation in Malaysian Mahseer, *Tor* sp., Broodstock Development Programme

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E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2023.36.4.004

Abstract

Malaysian Mahseer (*Tor* spp.) is a highly priced native freshwater fish in Malaysia and much sought after as food, ornamental and recreational fish. However, the production of mahseer is still insignificant. Due to the decline in the wild stock population of mahseer, farmers are compelled to rely heavily on imported broodstock or hatchery-produced fingerlings. The R&D on mahseer is also limited with only several reports on breeding techniques, feed requirement and genetic distance between populations. The availability of superior breeding stocks, efficient hatcheries management and formulation of maturation diet that could expedite the maturation process could greatly assist in increasing mahseer aquaculture productivity. This paper presents the R&D carried out by the Fisheries Research Institute, Department of Fisheries Malaysia starting from the 11th Malaysia Plan (2016 to 2020), particularly on the broodstock development, breeding, disease, seed management, formulation and validation of maturation diet. In this programme, broodstocks obtained from five different states namely Pahang, Johor, Sarawak, Perak and Terengganu, Malaysia were used as a base population and mated using full diallel method to produce a total of 75 families. The maturation diet enhanced the breeding capacity and shortened the maturation period of mahseer from 3.0 years to only 1.8–2.0 years. Three innovations, hatching system, nursing system and maturation diet were produced from this programme were also registered innovations. This paper also deliberates the challenges faced in broodstock development programme and the way forward.

Keywords: Mahseer (*Tor* sp.), broodstock maturation, aquaculture, maturation diet, hatchery, nursery

Introduction

Mahseer (*Tor* spp.) is a highly priced indigenous freshwater fish in Malaysia. The fish is called "Empurau" or "Semah" in Sarawak or "Pelian" in Sabah (Mohsin and Ambak, 1991; Litis et al., 1997; Ng, 2004). This fish is part of an important group of freshwater cyprinids collectively known as mahseers that inhabit mountainous rivers and lakes of the Himalayan belt from Afghanistan to Indonesia and Myanmar. Mahseers form a significant stock of indigenous fish in India, Nepal, Bangladesh and Pakistan with potential for the freshwater aquaculture industry (Ramezani-Fard and Kamarudin, 2012).

The first mahseer species was described in 1822 (Hamilton, 1822) followed by many investigations on the taxonomy, nomenclature and systematics of the *Tor* genus. Nevertheless, some taxonomic ambiguity continues to exist (Pinder and Raghavan, 2013). In Malaysia, Mohsin and Ambak (1983) described *Tor tambroides* and *T. soro* as two valid mahseer species found in Peninsular Malaysia, while Ng (2004) suggested the occurrence of three (3) species i.e. *T. tambroides*, *T. tambra*, and *T. douronensis*. A recent review by Pinder et al. (2019) stated that from the 16 *Tor* spp., only *Tor tambra* (Cuvier and Valenciennes, 1842) and *Tor tambroides* (Bleeker, 1854) were found in Malaysia. Pinder et al. (2019) pointed out that topotypic *T. tambra* had been demonstrated to be genetically alike to mahseer populations found across the mainland of Southeast Asia, including in Malaysia which were recorded as *T. tambroides* (Walton et al., 2017). This observation is in accordance with propositions of Roberts (1993; 1999), who considered *T. tambroides* as junior synonym of *T. tambra*. However, materials of *T. tambroides* from Sumatra were found to be genetically distinct to all material of *T. tambra* from the Peninsular and Java (Walton et al., 2017) thus leading to the question of whether *T. tambra* and *T. tambroides* are identical is still not resolved. Thus, the taxonomic status of mahseer in Malaysia is unclear and needs to be re-evaluated. Therefore, it is classified as data deficient (Kottelat et al., 2018). More facts on the current status regarding the mitogenome and population genetics of Malaysian Mahseer was provided by Jaafar et al. (2021).

Mahseer with its large-body, remarkable appearance and exquisite taste fetches high market demand and value as food fish. In addition, it is also an exceptional game fish and much sought-after ornamental fish. Mahseer is regarded as one of the most expensive freshwater fish with a market price of around MYR800–MYR1,000 (USD170–USD215) per kg (Bernama, 2021) and more so for wild-caught mahseer (Ramazeni-Fard and Kamarudin, 2012).

With a great demand and value, successful induced spawning in captivity and ready acceptance of artificial feed, mahseer is considered a good prospect for aquaculture (Ng, 2004; Ingram et al., 2005). In Malaysia, production of mahseer was insignificant until several years ago when it gained much attention from local farmers (Ingram et al., 2007). Among the major challenges that discouraged intensive farming of this species were the slow growth rate and difficulty in gaining fry. Mahseer is a seasonal spawner or "asynchronous spawner" that is capable of spawning only several times per year with significant environmental stimuli (Nguyen et al., 2006; Ingram et al., 2007; Ismail et al., 2011). This setback has led to uncontrolled harvesting of wild mahseer fry from the natural ecosystem, which is also against the national conservation strategy.

Significant degradation in natural habitats from anthropogenic activities (deforestation, agricultural development), pollution and climate change has also manifested the problem and contributed to the steady decline in the distribution and abundance of the wild mahseer population (Ng, 2004; Sungan et al., 2006). All these circumstances may lead to their extinction. Although, mahseer is not listed by the International Union for Conservation of Nature (IUCN) as a protected or endangered species, the drastic decline in natural populations has raised significant concern.

In view of the economic and conservation worth, a systematic mahseer broodstock development programme was commenced by the Fisheries Research Institute (FRI), Department of Fisheries (DOF), Malaysia in the 11th Malaysia Plan (RMK11, 2016–2020) as a potential new aquaculture species. This programme aimed to produce high-quality, fast growth strains and establish the hatchery technology for seed production. The reliance on imported seeds is risky because of the possible transmission of pathogens that could threaten the natural stock and sustainability of aquaculture industry. This article aims to complement the existing information on mahseer research in Malaysia, particularly the main components of the broodstock development programme which includes selecting potential broodstocks, estimating genetic distances, mating design, production of progenies, performance appraisal, improving hatchery and nursery practices, developing and evaluating maturation diets, disease occurrences, control measures and innovations developed under this programme. The challenges and prospects for future development are also discussed. This review is anticipated to identify gaps in mahseer research and development in Malaysia and help to intensify mahseer farming industry and conservation efforts.

Mahseer Production

The production of mahseer in Malaysia from 2010 to 2022 is presented in Table 1 (Department of Fisheries Malaysia, 2022). Most of the production is through inland capture and has been gradually decreasing over the years. The states of Kelantan and Sarawak are the two major producers. The highest production (71.32 tonnes) was recorded in 2010 through inland capture. The production drastically declined to 59.64 tonnes in 2011 (more than 90 % decrease) and has continued to decline since then. The production of mahseer from aquaculture activity started to be listed in the DOF statistics in 2012 but the volume was very small i.e., only around 15.0 tonnes. The latest total mahseer production from inland capture and aquaculture is only 4.29 tonnes.

Table 1. The production of mahseer, *Tor* sp. from 2010 to 2022 disclosed in the Annual Fisheries Statistics (Department of Fisheries Malaysia, 2022).

Year	Production (tonnes)	
	Inland capture	Aquaculture
2010	713.20	No record
2011	59.64	No record
2012	24.40	15.00
2013	25.05	19.66
2014	29.43	18.05
2015	29.00	24.70
2016	52.39	18.03
2017	26.21	8.19
2018	14.39	12.31
2019	6.21	3.54
2020	11.97	22.72
2021	17.41	14.84
2022	47.78	4.29

Seed production and culture were initiated to reduce the pressure on mahseer fisheries. Several hatcheries were established in Sarawak and the Peninsular Malaysia. In the Peninsular, mahseer seeds were initially produced by three government hatcheries i.e., the Fisheries Research Institute (FRI) Glami Lemi, Negeri Sembilan; the Aquaculture Extension Centre (PPA) Perlok, Pahang; the PPA Bukit Tinggi, Pahang and later together with several private hatcheries (Fig. 1). According to the latest statistics, a total of 3,750 mahseer seeds were supplied from the government hatcheries and 237,760 fry were recorded from private entities which could also be obtained from the wild (Department of Fisheries Malaysia, 2020). This number is still small to cater for the demand of the aquaculture industry.

Research on Mahseer Breeding and Artificial Spawning

In Malaysia, the Fisheries Research and Production Centre (IFRPC), Department of Agriculture Sarawak was among the pioneers involved in mahseer R&D. The first successful induced ovulation of *T. tambroides* in captivity was achieved by Ingram et al. (2005) and followed by grow-out evaluation trials (Ingram et al., 2006). The knowledge on the reproductive biology of these species and their breeding performance in captivity were strengthened in the later years (Ingram et al., 2007). All these information and technologies spurred the advance in large-scale production of *Tor* spp. in Sarawak for aquaculture and conservation efforts through genetically sound stock enhancement programmes.

Meanwhile in the Peninsular Malaysia, the first commercial breeding occurred in 2006 at the PPA Perlok, Jerantut, Pahang (Ramezani-Fard and Kamaruddin (2012). Females were successfully spawned with hormonal administration, and about 5,000 eggs were obtained. After 72 hours, 4,500 larvae were collected. Later, Azuadi et al. (2011) reported on the enhancement of breeding performances of domesticated mahseer female broodstock using commercial synthetic hormone (salmon gonadotrophin releasing hormone analogue) and embryonic and larval development from the induced ovulation (Azuadi et al., 2013). The latest endeavour on *Tor* sp. research focused on hybrid development (Azahar, 2019; Azfar-Ismail et al., 2020).

Mahseer broodstock development by the Fisheries Research Institute

Although more than 10 years since achieving the first commercial breeding, mahseer seed production is still very low. Under the 11th Malaysia Plan, a systematic mahseer broodstock development programme was initiated to mitigate the problem. The R&D activities implemented included genetic variation determination, selective breeding, rearing, performance evaluation and maturation feed formulation. The goals of the programme are: (a) to improve the induced breeding technique and nursing procedure, (b) to develop faster growing mahseer varieties by genetic improvement, (c) to support the private hatcheries in producing good quality mahseer fry.

Establishment of baseline broodstock population

Selection of the founder stocks (P_0)

Starting from the year 2016, the founder stocks were obtained from five different locations; Sungai Jerai, Pahang (PHG); Sungai Kejar, Gerik, Perak (GPRK); Sungai Berang, Hulu Terengganu, Terengganu (TGN), Sungai Anak Endau, Mersing, Johor (MSJ) and Hulu Sungai Rajang, Sarawak (EMS). The PHG, GPRK and TGN founder stocks were collected from the wild at juveniles' stages and were domesticated in ponds at the FRI Glami Lemi (FRI GL), Negeri Sembilan, Malaysia until matured. The stocks from MSJ and EMS were procured from local fish suppliers. The selected broodstocks comprised of 95 individuals as elaborated in Table 2.

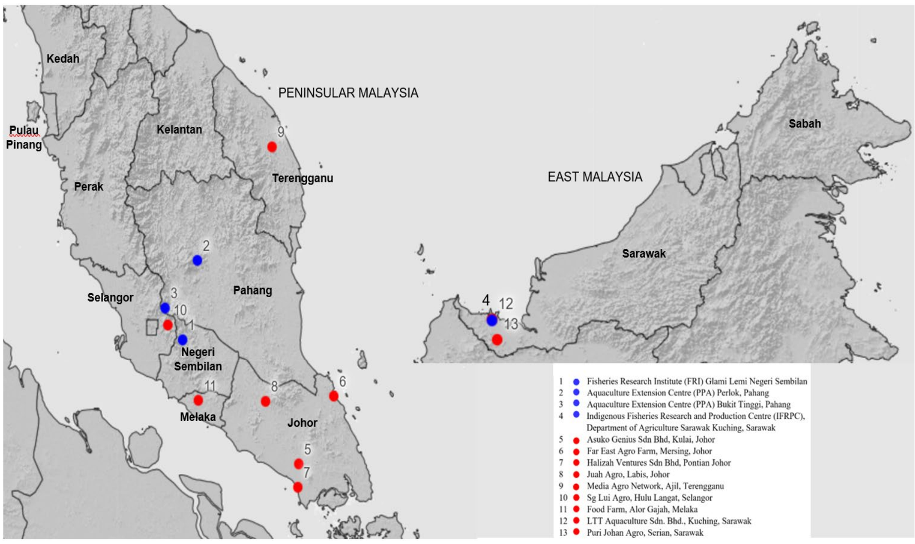

Fig. 1. Location of government (●) and private (●) mahseer (*Tor* sp.) hatcheries in Malaysia.

Table 2. Number of males and females and average body weight (± SE) of the mahseer (*Tor* sp.) founder stock.

	Pahang	Perak	Terengganu	Johor	Sarawak
Total (piece)	30	25	20	15	5
Male	10	10	5	5	2
Female	20	15	15	15	3
Body weight (kg)	1.25 ± 0.45	0.9 ± 0.73	1.2 ± 0.44	0.8 ± 0.36	3.0 ± 0.28

Transportation of broodstocks

The transport of broodstocks from supplier premises and wild habitat to the FRI GL involved long hours and confined space. Thus, the management and transportation of broodstocks were closely monitored to minimize stress. The broodstocks were starved for 1 day prior to transport and packed using special containers consisting of four layers of plastic with black plastic as the last layer to curtail fish aggressiveness. Oxygen was injected into the packaging to maintain and stabilise the oxygen supply.

Upon arrival, the broodstocks were weighed and the sexes identified. The sex was confirmed through abdomen hand stripping and the presence of tubercles on the operculum area. Morphological identification was done using keys provided by Inger and Chin (2002), Mohsin and Ambak (1983) and Kottelat et al. (1993). Prior to stocking, fin samples of individuals from each population were clipped for DNA analysis. The broodstocks were individually tagged with a Passive Integrated Transponder (Trovan, UK) tag with a unique alpha-numeric code. The tag was injected intramuscularly in each individual using hand-held injection devices (Trovan, UK).

Quarantine procedure

An important source of pathogens in aquaculture facilities is the new fish stock. Hence, the broodstocks must be quarantined to ensure that pathogens are not introduced into the hatchery. The broodstocks were held in 255-tonnes circular fibre recirculating aquaculture system (RAS) quarantine tanks with a capacity of 1 kg of fish per tonne of water. The broodstocks were physically examined for any unusual signs or symptoms of disease. Individuals from each population were screened for parasites and treated with sodium chloride (1.5 ppt) and formalin (25 ppm). The treatment was repeated every three days, with the water in the reservoir being completely emptied and replaced with fresh water on the 4th day of treatment. At the same time, salt and formalin were added in equal quantities. This procedure was repeated every 21 days. These parents were not disturbed unnecessarily because otherwise it could affect their feeding and the fish would refuse to eat for up to two weeks.

After 21 days of quarantine, the broodstocks were acclimatized in 5-tonne indoor RAS tanks with a stocking density of 1 fish per metric tonne. The broodstocks were fed *ad-libitum* with commercial pellet (34 % protein, 6 % lipid) twice daily until satiated. These broodstocks were physically checked monthly to see the growth rate of their reproductive systems. Morphological observations of the female broodstocks abdomen were used as indicators to determine the level of its maturation. The observations of enlarged and stiffening of abdomen tissue plus the reddish appearance of genitals often indicated that the female broodstock was ready to lay eggs.

Water quality

Physical water quality parameters such as dissolved oxygen, temperature and pH were monitored using water quality multiparameter probe (YSI, USA) routinely throughout quarantine, rearing and spawning. The 5-tonne RAS tanks used for mahseer broodstock were equipped with a physical and biological filter system. Table 3 listed the average main water quality parameters recorded throughout the process. Our data is comparable to the water quality parameters for successful *Tor* spp. culture as listed by Rachmatika et al. (2005) that is similar to water quality in the natural ecosystem. Meanwhile ammonia and nitrite were monitored using multi-parameter water quality kit (Hach, USA) every five days. The tanks were cleaned and siphoned every three days by changing 80–100 % of the water. The tanks were refilled carefully with clean water so as not to stress the fish.

Table 3. The average (± SE) water quality parameter readings in *recirculating aquaculture system* (RAS) tanks used for mahseer, *Tor* sp. broodstock quarantine.

Water quality parameter	Readings
Temperature (°C)	24.35 ± 0.06
pH	7.11 ± 0.03
Dissolved oxygen (ppm)	7.92 ± 0.16
NH_4 (mg.L^{-1})	0.23 ± 0.04
NO_2 (mg.L^{-1})	0.18 ± 0.02

Determination of genetic distances and genetic population structure

For artificial propagation and conservation purposes, it is crucial to compare the genetic diversity of the broodstocks to those of the wild populations (Liu et al., 2018). This is because, genetic diversity enhances the sustenance of species by making it adaptable to shifting environmental conditions (Ye et al., 2022). According to Gjedrem et al. (1991), capturing a broad variety of genetic diversity at the beginning of a breeding programme could lead to success in genetic improvement and greater chances for genetic improvement of important traits (Loughnan et al., 2016) and avoid genetic diversity loss that could occur in closed populations due to genetic drift and inbreeding (Frankham et al., 2002). Hence, our first activity in this breeding programme was to establish and validate the genetic diversity and distances between the mahseer's founder stock populations.

Genetic diversity, population structure, genetic relatedness, and demographic aspects between populations of founder stocks selected for this programme and several other broodstocks from 11 locations in Malaysia were characterised and genotyped by Chew et al. (2021) using 22 microsatellite (SSR) markers. The microsatellite primer pairs, fourteen (NY01-NY14) were designed specifically for *T. tambroides* following Nguyen et al. (2006), while eight (BS02-BS9) were designed from the microsatellite DNA sequences of *T. tambroides* (Chew et al., 2021). Table 4 shows the genetic diversity and polymorphism of the five mahseer founder stocks based on 22 microsatellite markers. The highest allelic richness (Ar) was seen in the Johor (MSJ) population (5.000) and the lowest in the Sarawak (EMS) population (1.2727). The number of effective alleles (Ae) produced varied from 1.273 to 3.160, with the highest recorded in the population of Terengganu (TGN) and the lowest in the population of Sarawak (EMS) (1.273). A total of 19 private alleles unique to a single population were found in the *Tor* samples, with the highest number in the TGN population (9 alleles), followed by the Perak (GRPK) and MSJ populations (3 alleles). The percentage of polymorphic loci ranged from 27.27 % in the EMS population to 95.45 % in the TGN populations. The lowest genetic variation was in the EMS population, which might be attributed to the small sample size. The highest gene diversity He (0.4506), observed heterozygosity Ho (0.4513), and polymorphism information content PIC (0.4354) were found in TGN, while the lowest He (0.0682), Ho (0.2727), and PIC (0.1023) were found in EMS. PHG, GPRK, and EMS were in HWE (Ho > He and negative in FIS value). Heterozygote deficit (He > Ho) occurred in MSJ, while inbreeding (positive FIS value) was detected in TGN and MSJ populations. However, the deviations from HWE were not significant ($P > 0.05$) in all five founder stock populations.

Pairwise Nei's genetic distance between founder stocks ranged from 0.153 to 0.351, as shown in Table 5 (from Chew et al., 2021). The lowest value of genetic distance (0.153) was found between populations of TGN and MSJ, indicating that these two founder stocks were genetically most closely related. The populations of MSJ and GPRK (0.154) also had a low genetic distance. The highest value of genetic distance (0.351) was found between the populations of EMS and PHG. A high

Table 4. Genetic diversity and polymorphism of mahseer (*Tor* sp.) founder stock populations by microsatellite genotyping using 22 SSR markers from different states of Malaysia.

State	N_A	A_r	MAF	Ae	N_G	A_p	% Polymorphic loci	He	Ho	Fis	PIC	HWE p-value
Perak	85	3.8636	0.6591	2.948	4.7273	3	77.27	0.4081	0.4176	-0.003	0.3831	0.3141
Pahang	60	2.7273	0.6736	2.226	3.1364	2	63.64	0.3676	0.4504	-0.203	0.3395	0.3443
Johor	109	5.000	0.6412	2.729	6.1818	3	77.27	0.4282	0.3458	0.145	0.3994	0.1805
Terengganu	103	4.6818	0.6234	3.160	5.0000	9	95.45	0.4506	0.4513	0.023	0.4354	0.3796
Sarawak	28	1.2727	0.8636	1.273	1.0000	2	27.27	0.0682	0.2727	-1.000	0.1023	1.000
Mean		3.5091	0.6922	2.4672	4.0091		68.18	0.3445	0.3876		0.3319	

NA – total allele number, A_r – allelic richness, MAF – major allele frequency, Ae – number of effective alleles, NG – number of genotypes, A_p – private alleles, He – expected heterozygosity, Ho – observed heterozygosity, Fis – inbreeding coefficient, PIC – polymorphism information content, HWE (Hardy-Weinberg Equilibrium) p-value – Exact test for HWE using a Markov Chain for all locus.

Table 5. Pairwise differentiation (F_{ST}) between mahseer (*Tor* sp.) populations (Weir and Cockerham, 1984) (above the diagonal) and Nei's (1983) genetic distance coefficient (below the diagonal) of the five studied mahseer founder stock populations used in this programme (extracted from Chew et al., 2021).

Population ID	Sarawak (EMS)	Perak (GPRK)	Johor (MSJ)	Pahang (PHG)	Terengganu (TGN)
Sarawak (EMS)	-	0.112*	0.072	0.227	0.104
Perak (GPRK)	0.276	-	0.092*	0.210*	0.136*
Johor (MSJ)	0.275	0.154	-	0.150*	0.061*
Pahang (PHG)	0.351	0.293	0.261	-	0.126*
Terengganu (TGN)	0.321	0.223	0.153	0.258	-

*Indicates a significant difference at $P < 0.05$.

genetic distance was also found between the populations of EMS and TGN (0.321) and between GPRK and PHG (0.293). In general, the EMS and PHG populations had higher genetic distances (0.258–0.351) when these two populations were compared with all other founder stock populations. On the other hand, pairwise differentiation between founder populations (F_{ST}) ranged from 0.061 to 0.227. High population differentiation with a pairwise $F_{ST} > 0.2$ was observed between populations of EMS and PHG (0.227) and between populations of GPRK and PHG (0.210). The population differentiation was significant ($P < 0.05$) between populations of GPRK and EMS, GPRK and MSJ, GPRK and PHG, GPRK and TGN, MSJ and PHG, MSJ and TGN, and PHG and TGN. The gene flow between populations was low in the differentiated populations.

The selected mahseer founder stocks were successfully characterized using microsatellite markers. In general, the mahseer founder stocks still exhibit sufficient genetic variation (mean allelic richness Ar 3.5091, mean polymorphic loci 68.18 %, mean PIC 0.3319), were weakly differentiated, and did not interbreed (low genetic admixture, low gene flow across all loci (Chew et al., 2021), suggesting that the selected founder stocks were suitable as breeding strains for the mahseer breeding program for aquaculture development. The results of this study differ slightly from those of Esa et al. (2008), Esa and Rahim (2013), and Esa and Rahim (2013), who studied the same species using specimens collected in the 2000s, with study sites slightly different from those of the present study. They found no significant genetic differences between *Tor* spp. in Peninsular Malaysia, but significant genetic differentiation was observed between *Tor* sp. populations in Peninsular Malaysia and Sarawak based on maternally inherited genes (CO1) and SSR markers.

Development of the base population (P_1)

Before crossing the founder population, the male and female broodstocks were stocked in 25-tonne tanks. The mahseer broodstocks were mated within and between populations to produce purebreds and crossbreeds in a full-diallel cross design (Table 5). Ten pairs of broodstocks were mated at a ratio of one male to one female (1:1) in each cross. Sexually matured broodstocks were identified based on the enlargement and reddish of cloaca as well as the softness of abdomen. Salmon gonadotropin releasing hormone (Ovaprim, Syndel, USA) was used to stimulate the egg production. The Ovaprim dosage used for the female broodstock was 0.6 mL per bodyweight and 0.3 mL per bodyweight for the male broodstock. The hatching and breeding of each family were carried out in separate tanks according to the procedures and systems that had been developed by the FRI GL which enabled the hatching rate to increase by up to 90 %. The cross-breeding activity was carried out over 12 months and a total of 83 families (P_0) were produced (Table 6).

Seventy-five families for the base population were successfully produced in this programme. However, there was a problem with the production of breeding stock from Sarawak due to limited broodstock sources, while the stock from Terengganu as the seeds by females broodstock were not feasible anymore for fertilization due to over maturation.

Growth performance of base population

The growth performance of the base population (particularly the length and bodyweight) from February 2018 until April 2019 involving 75 families is shown in Table 7. Sampling was done when the fingerlings were 1 inch in size. The fingerlings were fed with commercial starter pellets (42 % protein, Cargill, USA) for 3 months during this study. After that period, the fingerlings were fed commercial pellets (32 % protein, Cargill, USA) for 11 months. The results indicate that the Johor × Pahang population recorded the highest average bodyweight of 202 g and total length of 22.38 cm. The lowest average bodyweight value was recorded for the Pahang × Perak family of 37.71 g and a total length of 15.67 cm.

Upon reaching the targeted size of 2.0 cm, the fry was relocated to the FRI GL bio-secured Broodstock Multiplication Centre. They were reared in in-door tanks until they reached an average weight of 100.0 g. A morphological examination was conducted to eliminate any abnormalities such as sclerosis and deformities. About 50 selected potential broodstocks from each family were tagged and transferred to 30-tonne grow-out rectangular tanks until they attained the matured size of more than 1.2 kg. Body traits (total length and body weight) and reproduction status, including the reproductive hormone level, were recorded.

Table 6. Full diallel cross for mahseer (*Tor* sp.).

Female Male	Pahang P	Terengganu T	Perak PK	Sarawak S	Johor J
Pahang-P	PP (6)	PT (0)	PPK (6)	PS (3)	PJ (5)
Terengganu-T	TP (4)	TT (3)	TPK (5)	TS (0)	TJ (2)
Perak-PK	PKP (6)	PKT (1)	PKPK (5)	PKS (1)	PKJ (6)
Sarawak-S	SP (2)	ST (1)	SPK (1)	SS (3)	SS (2)
Johor-J	JP (5)	JT (0)	JPK (4)	JT (0)	JJ (4)

Number in the bracket shows the number of families produced.
The total families produced 3 × 15 crosses = 75 families, = 50 fish/families = 2250 fish.

Table 7. Total length and bodyweight (± SD) of mahseer, *Tor* sp. base population from 11 family crosses.

Families	Total length (cm)	Body weight (g)
Johor × Pahang	22.38 ± 1.3	155.65 ± 4.4
Johor × Johor	22.27 ± 2.2	142.63 ± 3.3
Perak × Johor	19.29 ± 1.1	103.18 ± 4.9
Perak × Pahang	18.11 ± 1.4	82.51 ± 3.2
Perak × Terengganu	18.92 ± 1.7	92.44 ± 2.6
Pahang × Perak	20.42 ± 3.2	120.78 ± 1.7
Pahang × Pahang	20.34 ± 2.1	118.90 ± 3.2
Pahang × Perak	15.67 ± 1.9	37.71 ± 6.1
Johor × Johor	22.27 ± 2.3	142.63 ± 2.7
Perak × Johor	19.29 ± 1.3	103.18 ± 4.1
Perak × Pahang	18.11 ± 1.5	82.51 ± 2.1

Throughout the 11th Malaysia Plan, 75 families were successfully produced from the intended target of 250 families and kept in separate tanks accordingly. A total of 50 individuals of each family population were selected, individually tagged and further grown up until matured. This programme is being continued in the 12th Malaysian Plan.

There is very little information on whether the breeding and culture trials for *Tor* spp. in India, Nepal or Bangladesh have resulted in commercialization for either food or conservation aquaculture, or even stock enhancement and ranching (Shrestha, 2002; Gurung et al., 2002; Rahman et al., 2005, Pinder et al., 2019.) Thus, it is difficult to compare our findings with others. This must be mainly caused by the slow growth performances of this species especially, while cultured in captivity. The limited availability of this species to Asian countries, particularly Southeast Asia, further limits research efforts. Correlation with other similar species such as "temoleh" golden carp (*Probarbus jullieni* Sauvage, 1880), "lampam" tinfoil barb (*Barbonymus schwanenfeldii* (Bleeker, 1854)), "sebarau" hampala barb (*Hampala macrolepidota* Kuhl & van Hasselt, 1823) was also impossible as there are no published records about the breeding and culture trials of these species.

Mahseer hatchery and nursery management

Newly hatched fish larvae are delicate and greatly affected by the culture conditions and handling (Hickey, 1979) and subjected to high mortality rate. Thus, knowledge on larval developmental stages and the behaviour of fish is extremely important and must be fully understood and closely monitored for successful larviculture (Kocovsky et al., 2012).

The production of mahseer fry to accommodate the aquaculture industry is a great challenge because of the low hatching rate (30–40 %). The common method of hatching or incubating mahseer eggs practiced is by scattering the eggs on a tray or the substrate which may cause injury to the eggs and leading to a low hatching rate as well as fungal infection. Hence an improvised hatching system was developed to increase the hatching rate. This system was designed to carry out the incubation process for mahseer and may also be used on other freshwater fishes. Comprising of five jars, one UV lamp and one pump per set and based on recirculating aquaculture system, this innovation is capable to accommodate up to 6000 eggs at a time. The benefits of this system include space saving, portable, easy to operate and increase in hatching rate from 40 % up to 90 %. This system was submitted to be registered with Malaysia Intellectual Property Office (MyIPO) as copyright of FRI with the application number CRLY00028241 in September 2022 under the title Mahseer Hatching System.

In order to ensure that the process of producing quality and sufficient seeds runs smoothly, FRI GL has established a system to nurse mahseer fry from hatching until 1–2 cm long. The fry would easily perish at these stages due to imperfect morphological condition. This system was designed to ensure a high survival rate of fry (up to 90 %) compared to the traditional method, where fry was immediately transferred and nursed in ponds and usually resulted in low survival rate (around 60–70 %) due to the presence of predators and limited source of feed directly added to the rearing pond. This system can accommodate up to 40,000 fries in one batch. Each batch will be nursed for two months or until it reaches about 2.0 inches during which they were fed *ad-libitum* with live feed (*Moina* sp.) and Aquaxcel™ micro pellet (Cargill, USA). Another advantage of this system is that it occupies a smaller area compared to ponds and is thus more cost effective. This system was registered with MyIPO as copyright of FRI (registration no. LY00028241) in January 2021 under the title Mahseer Nursery System.

Development of Mahseer Maturation Diet

The R&D on mahseer diet and nutrition in Malaysia is fairly extensive as documented by Redhwan et al. (2022). The general facts on dietary requirements of mahseer were provided by Ng et al. (2008), Miesieng et al. (2011) and Ng and Andin (2011). According to them, the major essential macronutrients were protein, lipid and carbohydrate and the recommended dietary protein content was in the range of 35–50 %. The effect of dietary lipid level on growth and muscle fatty acid composition of adult and fingerlings was reported by Ramezani-Fard et al. (2011), Ramezani-Fard et al. (2012) and Ramezani-Fard and Kamarudin (2012). More recent studies focused on the alternative feed ingredients and supplements used for mahseer (Kamarudin et al., 2012; Chowdhury et al., 2016; Ishak et al., 2016; Bami et al., 2017; Kamarudin et al., 2018; Asaduzzaman et al., 2019; Ishak et al., 2021; Kawata et al., 2021; Sulaiman et al., 2022; Farliana Wan Alias et al., 2023; Hossain et al., 2024). In contemporary aquaculture, probiotics supplemented diet is new avenue for enhancing growth performance of slow-growing fish. This was demonstrated by Asaduzaman et al. (2019) where host-derived probiotics, especially *Alcaligenes* sp. AFG22 and *Bacillus* sp. AHG22 were found to have significant potential as autochthonous probiotics for the stimulation of growth performance of slow-growing *T. tambroides*.

On the other hand, studies on the diet for faster maturation are very scarce. Thus, this scope of R&D was given priority in the broodstock development programme as inconsistent breeding period, seasonality and long duration of attaining maturation affect mahseer breeding (Ingram, 2005; Ismail et al., 2011). Azuadi et al. (2011) and Ingram et al. (2005) suggested that the matured domesticated mahseer broodstock normally weigh about 2.5–2.78 kg and ~2.5–3.0 years old. These features are not encouraging for aquaculture exploitation. Furthermore, there are no specific commercial maturation diets for freshwater fish, including mahseer.

The development of a maturation diet was initiated in 2016 with the inclusion of arachidonic acid (ARA) as one of the special ingredients in the diet formulation with the aim of increasing breeding capacity and efficiency. ARA has been demonstrated to be an important nutritional factor for fish reproduction (Sargent et al., 1999; Furuita et al., 2003; Pérez et al., 2007; Tocher, 2010). It is the main precursor of eicosanoids that include, among others prostaglandins (PGs) and thromboxanes (Tocher, 2003), which are essential in related physiological processes such as ovulation and implantation of the embryo (Smith, 2001). In fish, PGs have been shown to stimulate ovarian and testicular steroidogenesis and follicle maturation and trigger female sexual behaviour and milt increase in males (Kobayashi et al., 2002; Sorensen and Stacey, 2004).

Feeding trials

This study was conducted for nine months in RAS tanks. A total of 60 female mahseer broodstock individuals (aged around 16 months) from the same stock were randomly stocked into three 5-metric tonne RAS tanks. The fish were fed with three types of diet, (i) diet developed by FRI GL supplemented with beef liver flour containing ARA (mahseer maturation, KM1), (ii) comparative diet (mahseer maturation, KM2) and (iii) commercial diet (control) (Cargill, USA) for nine months. The KM1 and KM2 diets were formulated and processed using an extruder machine to produce floating pellets. The proximate and ARA contents of the diets are presented in Table 8.

Table 8. The proximate and arachidonic acid contents of the mahseer (*Tor* sp.) maturation diets used in the study.

Proximate contents (%, dry weight basis)	KM1 diet	KM2 diet	Control diet
Dry matter	90.04	90.36	91.29
Moisture	9.96	9.64	8.71
Crude protein	34.84	34.06	34.28
Crude fatty acids	9.41	9.22	9.27
Crude ash	4.82	8.16	8.27
Arachidonic acid (C20:4n6) (g per 100g)	1.11	1.25	0.03

KM: mahseer maturation.

After nine months, the weight of the fish ranged from 1.36–1.60 kg and certain percentage of the population reached maturity or in other words they were able to spawn. Mahseer broodstock fed with KM1 diet exhibit spawning capacity in 50 % of the population stock compared to mahseer fed with KM2 (28.6 %) and controlled diet (28.6 %). The fecundity (average number of eggs per kg of fish weight) was significantly higher ($P < 0.05$) in mahseer fed with KM1 (803 eggs kg^{-1}) and KM2 (789 eggs kg^{-1}) compared to the controlled diet (584 eggs kg^{-1})(Table 9). The number of eggs produced and gonad weight were also significantly higher ($P < 0.05$) in mahseer fed with KM1 and KM2 diets. In general, the relatively smaller average parent size reflects the earlier maturation age of fish as Roos et al. (2006) suggested. Our observation was different from Kunlapapuk and Kulabtong (2011) who reported bigger *Tor* sp. maturation size (around 3.8 kg) producing higher average of eggs which was 875 eggs kg^{-1} body weight.

An increase in PGE-2 hormone level was observed in mahseer blood serum after being given diet incorporated with ARA, although not significantly different ($P > 0.05$) than the control diet (Fig. 2). The results further confirmed that ARA may have stimulated maturation and mahseer breeding to some extent. The specially formulated maturation diet was able to shorten maturation age to around 1.8 to 2.0 years. Furthermore, mahseer fed with ARA enriched feed also demonstrated an increase in the reproduction cycle from two to five or more in a year. In some fish, the reproductive cycle was witnessed every month. The maturation diet formulation was registered with Malaysia Intellectual Property Organization (MyIPO) as intellectual property under the title "Fish feed composition for improving maturing phase of fish or aquatic animals" (COF No: 2019006209) and named as NutriKarp.

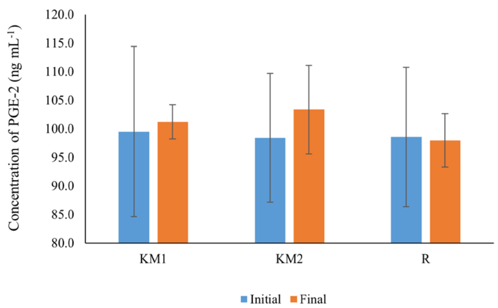

Fig. 2. Effect of three different diets on the PGE-2 hormone level in mahseer (*Tor* sp.) broodstocks.

Verification and validation of maturation diet

To further strengthen the initial findings at FRI GL, verification was conducted at two commercial hatcheries in Serian, Sarawak and Kulai, Johor. The findings revealed that 25 % of the tested mahseer stock in Sarawak and 10 % of tested mahseer stock in Johor were able to reach maturity earlier and successfully reproduced after nine months compared to the fish given control diet (Fig. 3).

In this programme, mahseer stock from Johor and Sarawak with weight around 0.72 kg and 1.94 kg respectively were able to spawn. This observation was differed from previously reported by Azuadi et al. (2011) and Ingram et al. (2007) which observed maturity at the size of 2.5 and 2.78 kg, respectively. Besides that, the body weight gain was significantly greater ($P < 0.05$) for the fish fed with Nutrikarp compared to the fish fed with control diet (Fig. 4). After nine months of feeding experiments, the fish fed with control group recorded a mean weight of 760.0 ± 84.1 g, while the fish fed with NutriKarp in Johor and Sarawak recorded a mean weight of 825.5 ± 94.0 g and 1943.0 ± 367.8 g, respectively.

Diseases of Domesticated Mahseer

The information on the prevalence of mahseer diseases was obtained from FRI GL own observations and in other farms including PPA, Perlok, Pahang and a private hatchery in Kulai, Johor. From our observation, domesticated mahseer were commonly infected with parasites, particularly *Piscinoodinium* sp. and *Ichthyophthirius* sp. Bacterial infection by *Aeromonas hydrophila* was also common in the nursery and grow-out stage. The reported mortality rates and suggested treatments are presented in Table 10. A parasite infection (Myxozoan) was reported in cultured mahseer in Tasik Kenyir, Terengganu, Malaysia (Székely et al., 2012). In Indonesia, *Lernea* sp. is common parasite attacking mahseer in cultivated ponds in addition to *Argulus* sp. and *Trichodina* sp. and also endoparasite fish tapeworm, *Bothriocephalus acheilognathi* (Muchlisin et al., 2014).

Issues and Challenges

There are already some encouraging achievements in

Table 9. Effect on reproductive parameters of mahseer (*Tor* sp.) broodstock fed with three different diets for nine months.

Reproduction parameters	KM1 Diet	KM2 Diet	Control Diet
Body weight (kg)	1.36 ± 0.11^{a}	1.52 ± 0.14^{b}	1.60 ± 0.02^{b}
Spawning capacity (%)	50.0^{a}	28.6^{b}	28.6^{b}
Fecundity (Number of eggs kg^{-1} body weight)	803^{a}	789^{a}	584^{b}
Gonad weight (g)	14.9 ± 1.3^{a}	14.6 ± 1.9^{a}	5.6 ± 1.7^{b}

KM: mahseer maturation.
Values are means ± SD. Values with different superscript letter are significantly different at $P < 0.05$.

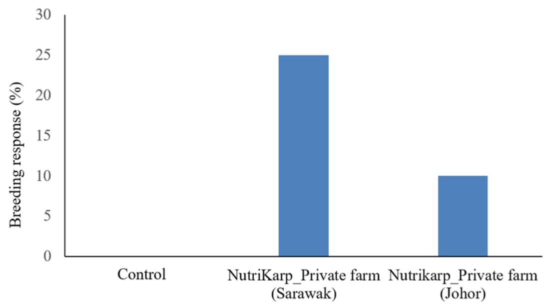

Fig. 3. Breeding response (%) of mahseer (*Tor* sp.) given different diets at two commercial hatcheries.

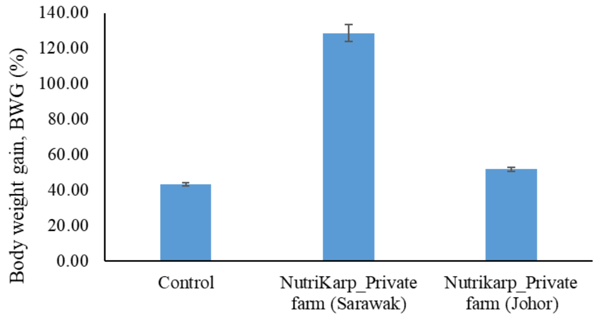

Fig. 4. Mean (± SD) growth performance (body weight gain) of mahseer (*Tor* sp.) given different diets at two commercial hatcheries

Table 10. Types of the pathogens and disease linked to domesticated mahseer, and the treatment methods practiced.

Types of pathogen (disease)	Mortality rates	Treatment
Parasite (*Piscinoodinium* sp.) (Velvet disease)	40 %-60 %	0.3-0.5 ppm copper sulphate ($CuSO_4$), one time in 3 days, 3 time continuously
Parasite (*Ichthyophthirius* sp.) (White spot)	40 %	25 ppm formalin and 1 ppt NaCl (salt), one time in 3 days, 3 time continuously
Bacteria (*Aeromonas hydrophila*)	55 %	SirehMax (Herbal preparation developed by Fisheries Research Institute). 100 ppm (incorporated into feed) 7–14 days of earliest feeding
Fungi (*Saprolegnia* sp.)	None	Methylene blue 0.5–1.0 ppm

mahseer broodstock development R&D programme at FRI GL. However, there are several significant challenges concerning the sustainability of this endeavour as mentioned below:

Broodstock resources

The broodstock development is a long term and continuous programme. One of the major problems faced is the difficulty in securing sufficient numbers of broodstocks with distinct genetic and phenotypic variation. Acquisition of broodstocks especially from Sarawak was very tedious especially with the full enforcement of the Sarawak Biodiversity Centre Ordinance (1997) which reflects the ordinance's effectiveness in conserving the Sarawak's biodiversity.

High operational cost

The broodstock development is a timely commitment which involves many resources (human, infrastructures, machinery, space, biosecurity system, utilities) hence making the programme very costly. Thus, the execution of this project requires significant funding which cannot be compromised.

Inadequate logistics

The availability of sufficient tanks and ponds is crucial to maintain mahseer broodstocks since the age of maturity is over 2 years. At FRI GL, there are limited numbers of spawning or rearing tanks to accommodate all families produced for performance test in tanks. Consequently, the number of families expected to produce in each generation of more than three was not always achieved. On top of that, FRI GL also has an on-going red tilapia hybrid broodstock development programme.

Clean water resources

Continuous supply of clean and unpolluted water is a must for this programme. At the FRI GL, the provision of clean water from Sungai Glami and Sungai Tinggi was frequently compromised, particularly during periods of heavy rainfall. A mixture of water and mud has the potential to impact mahseer stocks negatively and may even result in mortality.

Knowledge gaps

There is still a wide gap in our researcher's understanding and skills in executing this programme. A lot of pertinent information regarding our broodstocks is still unavailable and not well comprehended. During the egg release period, most broodstocks which are expected to reach maturity were found to have surpassed the ovulation period (over ripe). This crucial information must be established in order to have a successful breeding strategy. The industry also had to deal with the similar problem. Additionally, private hatcheries frequently lament the lack of skilled labour, which has impeded the operational flow of seed production.

Way Forward

In overall, the future of mahseer aquaculture is promising with good demand and great prices. Among the best quick win solution that could be adopted in increasing the current production of mahseer in Malaysia is to enhance close networking and constant technology transfer between FRI GL and private hatcheries. From our observation, the existing private mahseer hatcheries' knowledge and expertise on the culture aspects, broodstock and post hatchery management are up-to-date and adequate. With the help of improvised broodstocks from FRI GL and technical support in broostocks and hatcheries management, the existing hatcheries could produce better quality and quantity of mahseer seeds and fry. However, the awareness on mahseer diet needs to be improved. Since maturation diet (NutriKarp) developed by FRI GL was demonstrated to expedite maturity development in mahseer, commercialization of NutriKarp could help the industry to progress further. After the success of NutriKarp, FRI GL has also come up with specially formulated mahseer finisher diet. The initial findings suggest that the formulated feed is able to enhance the taste of cultured mahseer. The diet has been verified by a few private farmers and needs to be further verified and validated.

The challenge of procuring breeding stock, particularly from Sarawak was addressed through smart partnership with private mahseer producer and breeder in Sarawak and also local mahseer producers in the Peninsular Malaysia. In addition, the Standard Operating Procedures Manual is in the final steps of publishing for distribution to hatcheries, farmers and other interested parties. In addition, FRI GL is also providing active transfer of technology to main mahseer farmers and producers particularly in Johor and Terengganu.

Conclusion

The mahseer R&D programme by FRI GL has progressed into the 8 years of implementation and has produced some significant achievements. The selected broodstocks from five states in Malaysia were used as base population. The information on genetic variation and growth performance of P_0 has been collected and analysed. Because of the slow growth characteristics, the programme has only produced until F_0 with a total of 75 families from four batches. The maturation diet was able to enhance the breeding capacity and shorten the maturation period of mahseer from around 2.5–3.0 years to only 1.8–2.0 years. Three innovations were produced i.e., mahseer hatching system, mahseer nursing system and mahseer maturation feed. Due to the high commercial value of this species, this programme is being continued in the 12th Malaysian Plan.

Acknowledgements

This study was funded by the 11th Malaysia Plan Development Fund (No. 22501 037 0001) of the Fisheries Research Institute, Department of Fisheries, Malaysia. The authors would like to thank Mr Azwan Jaafar and Puan Najihah Mohamed from CMDV, MARDI. Our heartfelt gratitude also goes Dr. Hj. Zainoddin Jamari for his constant encouragement and guidance towards the execution of this project.

Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: Muhammad Zudaidy Jaapar: Conceptualisation, data curation, writing - reviewing and editing. Wan Norhana Md Noordin: Conceptualisation, writing - original draft preparation, reviewing and editing. Hanan Mohd Yusof: Data curation, writing - reviewing and editing, validation. Amatul-Samahah Md Ali: Writing - reviewing and editing. Chew Poh Chiang: Writing - reviewing and editing, validation. Azhar Hamzah: Conceptualisation, writing - reviewing and editing, validation.

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