

Toward Systematic Breeding of Asian Sea Bass, *Lates calcarifer* (Bloch, 1790), in Malaysia: Status, Challenges and Prospects for Future Development

SHAHARAH MOHD IDRIS^{1,*}, WAN NORHANA MD NOORDIN², FATIN OSMAN MANAH¹, AZHAR HAMZAH²

¹Fisheries Research Institute, Department of Fisheries, Tanjung Demong, Besut, 22000, Terengganu, Malaysia

²Fisheries Research Institute, Department of Fisheries, Batu Maung, 11960, Penang, Malaysia

© Asian Fisheries Society
Published under a Creative Commons

license

E-ISSN: 2073-3720

<https://doi.org/10.33997/j.afs.2022.35.1.001>

*E-mail: shaharah@dof.gov.my | Received: 14/09/2021; Accepted: 25/01/2022

Abstract

Asian sea bass, *Lates calcarifer* (Bloch, 1790), is one of the most farmed marine fish in Malaysia since the 1970s and is predominantly cultured at present. Although it has been farmed for some time, there is no systematic breeding programme in place. Local hatcheries rely heavily on wild and imported broodstock. However, imported stocks do not guarantee improved quality and even increase the chances of introducing pathogens into the country. Availability of superior breeding stocks, efficient dissemination and control of seed production could greatly assist in increasing Asian sea bass production. This paper presents the status of Asian sea bass production, its culture history in Malaysia and the current broodstock development programme implemented by the Fisheries Research Institute, particularly in the 11th Malaysia Plan from 2015 to 2020. In brief, broodstock from Malaysia, Thailand and Indonesia were used as base population and were mated using full diallel method to produce cohorts. Three pairs of the selected broodstock were used for each cross and spawning performance was evaluated. The growth performances of the first generation (F₁) offsprings were assessed in different culture systems. Physico-chemical water quality parameters such as dissolved oxygen, temperature, pH and salinity were maintained at optimal levels during all stages of the experiment. Results indicate that the growth performance of F₁ improved by more than 60 % in terms of weight gain compared with the base population. The project demonstrated the benefits of using selective breeding to enhance the production of Asian sea bass.

Keywords: stock improvement, genetic improvement, selective breeding, growth performance

Introduction

Asian sea bass, *Lates calcarifer* (Bloch, 1790), locally known as siakap putih is one of the key aquaculture species in Malaysia. They are fast-growing, hardy, easily domesticated to aquaculture conditions, tolerant to salinity variations (as a euryhaline species) and well adapted in highly turbid waters (Husin and Ali, 1996). In addition, Asian sea bass fetches high market prices and their fingerlings are artificially propagated in hatcheries hence readily available. In Kelantan, Pahang, and Terengganu on the east coast of Peninsular Malaysia facing the South China Sea and Perak, Penang and Kedah in the northern part of the Peninsular, which faces the Strait of Malacca, are the main states for Asian sea bass farming. The bulk of production comes from floating net cages because of

the low investment cost and these floating cages are easy to construct, manage and versatile (Buendia, 1997).

From the 90s, about 50–90 % of the total annual production of cultured marine fishes in Malaysia was dominated by Asian sea bass. Groupers, *Epinephelus* spp., and John's snapper, *Lutjanus johnii* Bloch, 1792, and mangrove red snapper, *Lutjanus argentimaculatus* (Forsskål, 1775), represented about 5–12 % and 4–30 %, respectively (Hussin and Ali, 1996). At present, Asian sea bass is leading with about 55.3 % of the total marine fish cultured (17,263.61 tonnes out of 31,242.83 tonnes) (Department of Fisheries, 2019).

Figure 1 illustrates the trend in Asian sea bass

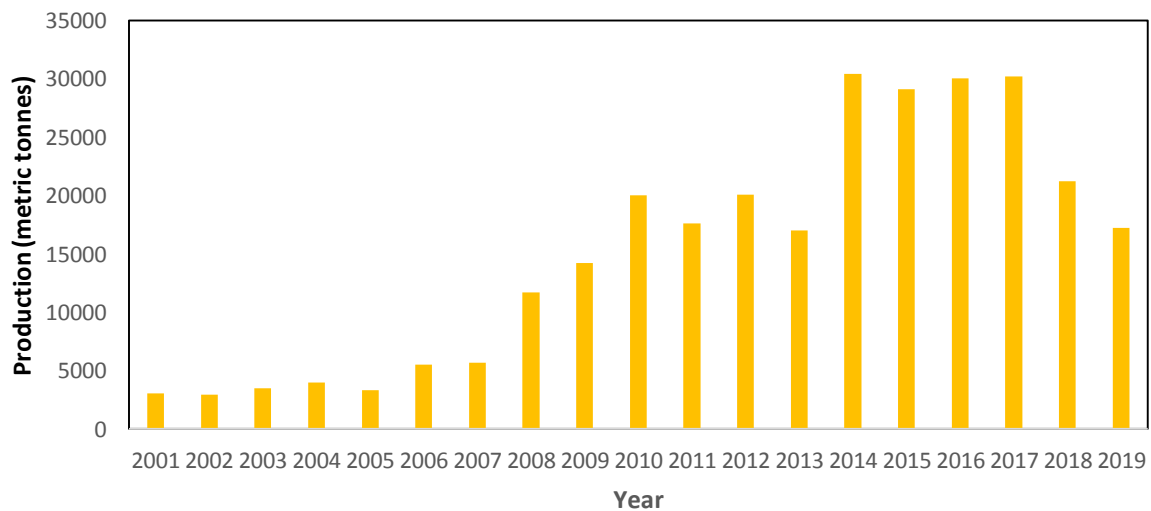


Fig. 1. Production of Asian sea bass *Lates calcarifer*, from 2000–2019 (Source: Department of Fisheries Malaysia, 2019).

production from the aquaculture sector in Malaysia. There was a gradual increase in production from the year 2000 until 2014 when it reached peak production at 30,440 tonnes (worth USD128 million). This could be related to the support from private hatcheries in producing large amounts of fish seed. However, production started to decline with only 17,263.61 tonnes (almost 50 % reduction) recorded in 2019 (Department of Fisheries Malaysia, 2019). The production decline could be due to several factors, including inconsistent supply of quality and adequate seeds, diseases such as iridovirus, viral nervous necrosis (VNN) and white spot (Kua, 2008; Ransangan et al., 2011; Chu et al., 2012; Ransangan et al., 2012; Szekely et al., 2013) and changing economic conditions (Aripin et al., 2019).

The Asian sea bass was identified as one of the priority species in the National Key Economic Area (NKEA), Economic Transformation Programme (ETP) under the agriculture sector in the year 2010 (World Bank, 2017). Under these programmes, the production from aquaculture was projected to improve through increased production, competitive culture practises and a knowledge-based approach. The Asian sea bass was chosen because its culture started way back in 1970s. The research and development on breeding, larval rearing and nursery technologies for Asian sea bass have been spearheaded by the Fisheries Research Institute (FRI), Department of Fisheries Malaysia (DOF) since the 1980s (Ali, 1987a; Ali, 1987b; Ali, 1987c; Hussin, 1987; Liong et al., 1988). Asian sea bass research and development was further strengthened in the 8th, 9th and 10th Malaysia Plans, spanning from 2001 to 2015. The research and development focused on broodstock development in the 11th Malaysia Plan (2016–2020). Although Asian sea bass has been farmed for over 50 years in the country, a systematic breeding programme was not in place. The local hatcheries relied heavily on wild and imported broodstock to produce seeds, with the bulk of the broodstock imported from neighbouring

countries, especially Thailand (Kechik, 1995). Using wild and imported broodstock does not assure high production yield because of unknown genetic backgrounds and low seed performance apart from the potential risk of introducing foreign pathogens. A small percentage of local hatcheries used domesticated broodstock produced through selective breeding as an alternative due to the high cost of imported broodstock.

However, the knowledge of hatchery operators on selective breeding was poor. These circumstances have driven FRI to focus on Asian sea bass broodstock development to help increase production in Malaysia by supplying high quality and improved broodstock to local broodstock multiplication centres. Similar initiatives have been established in Australia (Macbeth and Palmer, 2011), Singapore (Wang et al., 2006; Yue et al., 2009), Thailand (Joerakate et al., 2018) and Vietnam (Pham et al., 2018; Pham, 2019). Likewise, the challenges of the breeding programme for Asian sea bass in other countries were also focused on reproduction, larval rearing, and culture in captive environments. In most of the breeding programmes, quantitative genetic information (heritability and correlations) of traits was among the main activities conducted at the initial stage of the programmes (Wang et al., 2006; Macbeth and Palmer, 2011; Pham et al., 2018 and Joerakate et al., 2018).

This paper presents information on the production and culture of Asian sea bass in Malaysia, particularly the broodstock development programme implemented by FRI from 2016 to 2020 in the 11th Malaysia Plan. The paper also discusses the challenges and prospects for future development. Finally, the paper recommends roles and activities that should be implemented by those involved in the Asian sea bass broodstock development, dissemination and seed production management in Malaysia to boost the annual production of this species.

History of Asian sea bass culture in Malaysia

Marine fish culture in floating cages has rapidly developed since its introduction in the early 1970s, and significant expansion began in the late 1980s (Kechik, 1995). Asian sea bass is a major species of interest, with many grow-out farms in Malaysia practising polyculture with groupers and snappers in cages. According to Muhammad (1993), marine fish culture developed slowly because of inadequate supply of seed and low technological know-how in managing the cages.

Early R&D on Asian sea bass in Malaysia

The success in Asian sea bass propagation in Thailand has stimulated research in Malaysia. A unit responsible for research on hatchery propagation, larval feed development, and cage culture of Asian sea bass was established at the Fisheries Research Institute (FRI), Department of Fisheries Malaysia (DOFM), Penang. Larval propagation (using wild broodstock) was initiated by FRI in 1982 and the first spawning of sea bass broodstock raised in captivity was achieved in July 1985. A study on sea bass grow-out in coastal ponds was also carried out by the Brackishwater Fisheries Research Centre, Gelang Patah or presently recognised as FRI Gelang Patah, Johor. Later, the Marine Finfish Production and Research Centre in Besut, Terengganu or currently referred to as FRI Tanjung Demong (FRI TD) was set up in 1982 for marine fish fry mass production. The Asian sea bass fry production and culture methods are well-established in Malaysia.

In spite of a long history of aquaculture production, systematic and efficient breeding programmes to improve economically important traits in the farmed species have rarely been adopted. Selective breeding is a powerful tool to increase commercial production, and the success of selective breeding programmes depends on a systematic approach involving several steps, from the establishment of a base population to the formulation of the breeding objectives and selection strategies (Nguyen, 2016).

Development of Asian sea bass broodstock in Malaysia

Research and development activities under the broodstock development programme for Asian sea bass, including genetic variation determination, broodstock selection, fry production, rearing and performance evaluation, have been conducted mainly at FRI TD since 2016 with strong support from the government. The programme's goals are: i) to develop a faster-growing cultured sea bass through selective breeding, ii) to manage the genetic status of stocks, iii) to avoid inbreeding, and iv) to improve sea bass growth and survival to market size.

Establishment of Base Population

Selection of the founder stocks (P_0)

The parents of the base population (P_1) used in this breeding programme were obtained from three different locations: Thailand (Bangkok), Indonesia (Bali) and Malaysia (Terengganu and unknown). Males and females weighing 2.0 and 3.5 kg respectively were selected as founder stocks. Private hatcheries in Malaysia imported the parental stocks from Thailand and Indonesia. The Malaysia broodstock originated from the wild and was maintained in captivity for the breeding programme at the FRI TD hatchery since 2005.

The broodstock were held in 50-tonnes concrete tanks at the FRI TD hatchery. They were fed once daily with fresh fish (2–3 % body weight). The stocks comprised of 142 individuals as follows; Bangkok stock (20 males: 30 females); Bali stock (15 males: 24 females: 1 undetermined sex); Malaysia stock (Terengganu) (10 males: 14 females) and Malaysia (unknown) stock (10 males: 18 females). Since the bodyweight of all broodstock were in the range of 2.5–5.0 kg, the sex ratio was mostly female-biased. This is related to the protandrous hermaphrodite characteristic of Asian sea bass, wherein males change sex when they reach the total length and weight of about 60 cm and 3.2 kg, respectively (Dunstan, 1962).

Transport and quarantine procedure

The transport of broodstock from private hatcheries to FRI TD involves long hours of journey for large numbers of fish in a relatively small volume of water. Hence, packing, handling and transport of broodstock were closely monitored to minimise stress. The broodstock were starved for 2 days prior to transport and transferred in 100 L plastic drum supplied with aeration and filtered seawater. A coolant or ice pack was added to the drum to reduce the temperature to about 18 °C to sedate the fish. Oxygen was injected into the plastic drum to maintain and stabilise the oxygen supply.

Upon arrival, broodstock were weighed and the sexes identified. The broodstock were then transferred into 50-tonne concrete quarantine tanks with a maximum capacity of 2–3 kg of fish per tonne of seawater. Prior to stocking, fin samples of individuals from each population were clipped for DNA analysis. Treatment with 3 ppm acriflavine was executed for 24 h once the fish entered the hatchery to limit infection risk and facilitate their stress recovery. Individual sample from each population was screened for nervous necrosis virus (VNN) and iridovirus. In addition, bacterial and parasitic infections were checked on random samples.

During the 14-day quarantine period, the broodstock were fed twice daily with formulated feeds at 3 % of their biomass. On day 0 of the quarantine period, tank water was lowered to 2/3 of the tank depth, and 3 ppm acriflavine was added for 24 h. After 24 h (day 1), the seawater was changed (100 %), and 25 ppm formalin was added for the next 24 h with proper aeration to ensure that the dissolved oxygen content exceeded 4 ppm. The exchange of water and addition of formalin were repeated on days 2, 3, 4 and 5 if necessary. On the 6th day, 0.25 ppm organophosphate was added 24 h to treat the stocks for ectoparasite infections. On the 7th day, antibiotic treatment using oxytetracycline (0.5 g.kg⁻¹ feed) was initiated for five to seven consecutive days. Meanwhile, monthly treatment of 25 ppm of formalin for 24 h for three straight days was performed after the spawning season for prophylactic purposes.

Tagging

The broodstock were transferred to 20-tonne concrete stocking tanks after the quarantine period. In the stocking tanks, mature broodstock from each location were labelled with passive integrated transponders (PIT) tag attached to the fish's dorsal fin via the inter-muscle tagging method using hand-held injection devices.

Determination of genetic diversity

The ultimate success of a selective breeding programme depends largely on the P₀. Ideally, the P₀ should have high genetic diversity with large phenotypic variation. According to Gjerde et al. (2004), genetic evaluation should be the first step in establishing a breeding programme. In this programme, an assessment of genetic diversity of the Asian sea bass broodstock was carried out in collaboration with the Centre for Marker Discovery and Validation (CMDV), Malaysian Agricultural Research and Development Institute.

Fin clips of individuals were analysed for genetic diversity. About 20 mg of fin clip samples were added into a 50 mL tube, labelled with the fish tag number and kept in a liquid nitrogen container. According to manufacturer instructions, genomic DNA from the samples was extracted using Qiagen DNA extraction kit (Qiagen, Germany). The isolated DNA with a 10–20 µg yield with purity 1.8–2.0 was used for further PCR analysis. Twenty-six microsatellite loci described for Asian sea bass were used to analyse the population genetic structure. The loci were chosen based on high heterozygosity levels exceeding 70 % to enable DNA amplification. Microsatellites were amplified in a 10 µl final volume of PCR mixture containing 10× PCR buffer, 50 mM MgCl₂, 2 mM dNTP, 10 uM primers (5 µM forward, 5 µM reverse), 5 µM fluorescent-labelled primer, 5 U Taq Polymerase, and 20 ng of sea bass DNA. PCR conditions were set as follows: initial preheating at 94 °C for 30 sec, followed by 40 cycles

of 30 sec at 94 °C, annealing at the specific locus temperature (45–60 °C) for 45 sec and 45 sec at 72 °C, and final extension at 72 °C for 7 min. Genotyping after PCR process was performed for each individual by allele sizing on an ABI PRISM® 3700 DNA Analyser (Applied Biosystems, USA), using Hi-di formamide and the GeneScan™-500 LIZ® size standard (Applied Biosystems, USA) as an internal size standard. Allelic data were scored by Genemapper software.

Table 1 shows the genetic distance of three populations of P₁ stocks used in this programme. The genetic difference was observed between two populations within the total and two populations within the designated group. The results showed a large genetic distance between the parent from Bali and Malaysia (0.4938) besides Bangkok and Malaysia (0.3030). The results suggested that the parent from the three populations used in this programme contained high allelic and gene diversity. The present study's findings are consistent with Norfatimah et al. (2009), who reported genetic variation of Asian sea bass from Peninsular Malaysia based on the cytochrome b gene. According to Norfatimah et al. (2009), Asian sea bass from Terengganu possesses the highest variability with genetic distance among populations ranging from 0.000 to 0.260 compared to populations from Kedah, Melaka, Johor and Pahang. Whereas the Bali and Bangkok stocks demonstrated less variation (0.1029) and particularly the stocks from Malaysia (0.0640). Although the Asian sea bass populations from Bali and Bangkok were expected to be diverse because of the greater physical distance between the two countries, surprisingly, the Bali and Bangkok populations showed a common genetic make-up with spatial distant populations. The slight variation could be due to the movement of the fish species between Indonesia and Thailand. The findings are similar with Keenan (2000); Weiss et al. (2001) and Norfatimah et al. (2009), who suggested that although Thailand and Indonesia wild populations are highly structured with a limited gene flow as evidenced by a few populations, human intervention has tremendously globalised this highly commercial fish via primary and secondary translocations of Asian sea bass culture.

Development of the base population (P₁)

Before crossing the founder population, the male and female broodstock were stocked separated in 50-tonne tanks for 3 weeks. The broodstock were mated within the population to produce a purebred cohort (Table 2). Three pairs of selected broodstock were mated at a ratio of 1:1, in replicates and stocked in 10-tonne tanks for each cross. Spawning occurred at night, mainly between 8:00 and 9:00 pm for three to five consecutive days, starting from 2 to 5 days after the full moon. The floating, fertilised eggs were collected with a fine mesh scoop net in the morning. The eggs were washed repeatedly through a series of

Table 1. Genetic distance of Asian sea bass *Lates calcarifer* from three populations of founder stocks (P₀).

Population	Malaysia	Bangkok	Bali	Unknown
Malaysia	*****	*****	*****	*****
Bangkok	0.3030	*****	*****	*****
Bali	0.4938	0.1029	*****	*****
Unknown	0.0640	0.2653	0.3793	*****

***** - no value

Table 2. Mating of the Asian sea bass, *Lates calcarifer*, founder populations to produce purebred cohort.

	Female	Bangkok	Bali	Malaysia
Male				
Bangkok		Cohort 1		
Bali			Cohort 2	
Malaysia				Cohort 3

filters to remove algae and foreign materials before stocking them into the rearing tank. The eggs samples were placed in graduated cylinders for estimation of density. The number of eggs collected and fertilisation rate was recorded. Fertilised eggs were transferred into hatching and rearing tanks at a density of 50,000 eggs.m⁻³ (50 eggs.L⁻¹). Fertilised eggs from each cross were divided into two replicates rearing tanks with the same water parameter, tank management and feeding regime.

Growth performance evaluation of base population (P₁)

The hatched larvae of each cohort were reared until 2.5 cm and then transferred into nursery tanks. The first experiment on growth performance was carried out at a specific size of 40–200 g. The fish with an initial average weight of 40 g were stocked in 5-tonne tank (200 individual.tank⁻¹) with two replicates. The fish were fed pelleted feed twice daily at ratio of 3 % body weight in the morning and afternoon. The feed was weighed, and the total food consumed was recorded. A total of 30 fish were sampled monthly to measure the growth performance parameters, which included body weight (BW), total length (TL), standard length (SL) and body depth (BD). The length-weight relationship was plotted and the growth rate was measured and compared among replicate. The experiment was terminated when the average BW reached 200 g.

Data on growth, monthly percentage weight gain, weight gain per day, specific growth rate (SGR), feed conversion rate (FCR) and yields of the Asian sea bass reared in tanks are shown in Table 3. Asian sea bass fingerlings from Bangkok, Bali and Malaysia with an average mean weight of 52.52 g ± 6.80 g, 42.97 g ± 11.12 and 48.23 g ± 9.48 g respectively were stocked at 200 tank⁻¹ in different tanks in replicates. The fish BW reached an average of 267.86 g ± 37.54 in 120 days (95.17 % survival rate) for Bangkok strain, 234.65 g ±

52.42 g in 90 days (86.21 % survival rate) for Bali strain, 213.92 g ± 37.04 g in 150 days (99.00 % survival rate) for Malaysia strain. Sakaras (1987) demonstrated that stocking density of 100 and 300 m⁻³ in cages with larger sea bass juveniles of 16 cm and 60 g at initial size attained final body weights of 573.3 and 505.4 g, respectively in 7 months. Weight gain per day during the initial period ranged from 1.10 to 2.13 g per day, whereas the SGR ranged from 1.00 to 1.96 % per day. The results are comparable with previous reports where SGR was 5.6 % per day at 27 °C in 20 days' experiment using 1 g fish (Katersky and Carter, 2005). There was a linear relationship between SGR and fish weight where SGR increased as fish weight increased (Jobling, 1995). The final average size of sea bass at harvest (about 3 months) in this programme was above 200 g. This is in agreement with the growth of sea bass over 400 g in 6 months as reported by Schipp et al. (2007) and the growth recorded by Mackinnon (1989) whereby 45-day old fishes with a mean length of 50 mm grew to 300 mm (390 g) in 4 months.

Establishment of First Generation (F₁)

Production of the first generation (F₁)

The male and female broodstock were stocked separately in 50-tonne tanks for 3 weeks before mating. The first generation (F₁) was produced by 'diallel' crosses, as shown in Table 4. For this purpose, the broodstock selected based on the desired phenotype were PIT tagged and mated using full diallel method at a ratio of one male to one female in 10-tonne spawning tanks to produce the cohort.

Determination of genetic diversity

The determination of genetic diversity of F₁ was carried out by genetic distance and growth rate and the results are shown in Figure 2, which indicates the log probabilities [Ln P (D)] associated with different

Table 3. Growth performance of base population (P_1) of Asian sea bass, *Lates calcarifer*, from 40–200 g in tanks*.

	Bangkok	Bali	Malaysia
Rearing period (days)	120	90	150
Initial numbers (tails)	200	200	200
Final numbers (tails)	190	173	199
Initial body weight (g)	52.52 ± 6.80 ^a	42.97 ± 11.12 ^a	48.23 ± 9.48 ^a
Final BW (g)	267.86 ± 37.54 ^a	234.65 ± 52.42 ^a	213.92 ± 37.04 ^a
Weight gain (g.day ⁻¹)	1.79 ± 0.06 ^a	2.13 ± 0.15 ^a	1.10 ± 0.19 ^b
Specific growth rate (%.day ⁻¹)	1.36 ± 0.03 ^b	1.96 ± 0.42 ^a	1.00 ± 0.04 ^b
Survival rate (%)	95.17 ± 3.55 ^a	86.25 ± 3.18 ^b	99.00 ± 1.00 ^a
Feed conversion ratio	1.26 ± 0.02 ^a	1.27 ± 0.13 ^a	1.29 ± 0.02 ^a
Yield (kg.tank ⁻¹)	50.97 ± 1.71 ^a	40.36 ± 4.64 ^a	42.38 ± 7.53 ^a

*Values are means ± SD.

Mean ± SD in the same row with different superscript letters are significantly different ($P < 0.05$).

Table 4. Cohort diallel crossing of first generation (F_1) of Asian sea bass, *Lates calcarifer*.

Female \ Male	Bangkok	Bali	Malaysia
Bangkok	Cohort 1	Cohort 4	Cohort 5
Bali	Cohort 7	Cohort 2	Cohort 6
Malaysia	Cohort 8	Cohort 9	Cohort 3

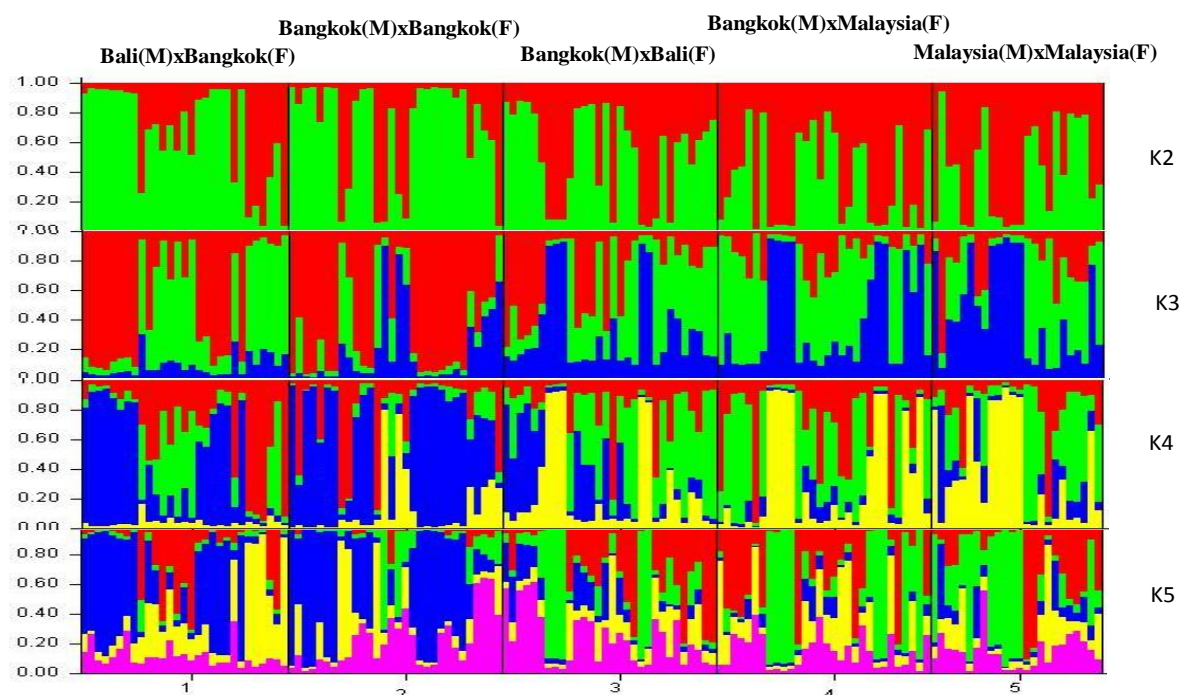


Fig. 2. Genetic content of first generation (F_1) of Asian sea bass, *Lates calcarifer*.

numbers of genetic clusters (K), which indicated the highest value at $K = 4$ [$\ln P(D) = -1074.5.7$]. According to the degree of admixture (alpha value), $K = 5$ gives

the nearest value to zero, indicating that the most appropriate number of genetic groups assigned for the given data set was five.

The analysis of 150 samples from five populations resulted in four populations with different genetic content. Out of the two populations, K4 (Bangkok × Malaysia) and K5 (Malaysia × Malaysia) were found to be in the same cluster with only a slight variation (0.0195) (Table 5). Based on the analysis, these populations were not used as they are likely to produce the same phenotypic features and have similar growth performances.

The results show that there was a variation between K1 (Bali × Bangkok), K2 (Bangkok × Bangkok) and K3 (Bangkok × Bali) populations. These results were significant with the results of the analysis performed on the base populations. The broodstock from Bali and Bangkok in 2017 (Table 1) showed a high genetic content and level of heterozygosity, thus making these two populations the best strains as candidates in the selective breeding programme. The reason for lower genetic diversity among F_1 is that the cultured stocks were already in their first generation of captive breeding. This observation is in accordance with Yue et al. (2009), who also noted that the reduction of genetic variation in cultured Asian sea bass stocks is common.

Growth performance of the first generation (F_1)

The growth performances of the F_1 fingerlings reared in 5-tonne fibreglass tank was measured in a similar method as mentioned above for P_1 . The result of F_1 growth performance was better for cohorts 4, 5, 6, 7 and 8 (Table 6) compared to cohort 1, 2 and 3 of P_1 (Table 3). Only eight cohorts were successfully produced despite the intended target of nine cohorts. The findings show that cohort 4 (Bangkok × Bali), cohort 5 (Bangkok × Malaysia), cohort 6 (Bali × Malaysia) and cohort 8 (Malaysia × Bangkok) reached a final average BW of $217.75 \text{ g} \pm 5.54$, $209.80 \text{ g} \pm 9.00$, $205.67 \text{ g} \pm 7.31$ and $201.07 \text{ g} \pm 0.14$ respectively at only 60 days. Whilst the final BW gained for cohort 7 (Bali × Bangkok) falls with an average of $215.48 \text{ g} \pm 7.85$ in 90 days.

In this study, the final average size of Asian sea bass was above 200 g at harvest, which is about 60 days. The rearing period recorded to reach 200 g in this programme was shorter than what was reported by Mackinnon (1989) and Schipp et al. (2007), wherein the growth of sea bass was noted to be over 400 g in 180 days and over 390 g in 120 days respectively. The

Table 5. Genetic distance of five populations of first generation (F_1) Asian sea bass, *Lates calcarifer*.

Population identification	Bali(M)x Bangkok(F)	Bangkok(M)x Bangkok(F)	Bangkok(M)x Bali(F)	Bangkok(M)x Malaysia(F)	Malaysia(M)x Malaysia(F)
Bali(M)x Bangkok(F)	*****	*****	*****	*****	*****
Bangkok(M)x Malaysia(F)	0.0687	*****	*****	*****	*****
Bangkok(M)x Bali(F)	0.0382	0.0781	*****	*****	*****
Bangkok(M)x Bangkok(F)	0.0627	0.1817	0.0316	*****	*****
Malaysia(M)x Malaysia(F)	0.0984	0.1436	0.0307	0.0195	*****

***** - no value; (M) - Male; (F) - Female

Table 6. Growth performance of first generation (F_1) of Asian sea bass, *Lates calcarifer*, from 40-200 g in tanks*.

	Cohort 4	Cohort 5	Cohort 6	Cohort 7	Cohort 8
Rearing period (days)	60	60	60	90	60
Initial numbers (tails)	200	200	200	200	200
Final numbers (tails)	200	199	200	199	200
Initial BW (g)	42.28 ± 0.31^c	72.08 ± 0.17^a	39.50 ± 0.00^e	43.12 ± 0.02^b	40.82 ± 0.31^d
Final BW (g)	217.75 ± 5.54^a	209.80 ± 9.00^a	205.67 ± 7.31^a	215.48 ± 7.85^a	201.07 ± 0.14^a
Weight gain ($\text{g} \cdot \text{day}^{-1}$)	2.92 ± 0.10^a	2.30 ± 0.15^{bc}	2.77 ± 0.12^a	1.92 ± 0.09^c	2.67 ± 0.003^{ab}
SGR ($\% \cdot \text{day}^{-1}$)	2.73 ± 0.05^a	1.78 ± 0.08^b	2.75 ± 0.06^a	1.79 ± 0.04^b	2.66 ± 0.01^a
Survival rate (%)	100.00 ± 0.00^a	99.25 ± 0.71^a	100.00 ± 0.00^a	99.25 ± 1.06^a	100.00 ± 0.00^a
FCR	1.24 ± 0.01^b	1.53 ± 0.04^a	1.24 ± 0.01^b	1.25 ± 0.01^b	1.25 ± 0.002^b
Yield ($\text{kg} \cdot \text{tank}^{-1}$)	43.55 ± 1.11^a	41.76 ± 2.09^a	41.13 ± 1.46^a	42.78 ± 2.02^a	40.21 ± 0.03^a

*Values are means \pm SD.

BW = Body weight, SGR = Specific growth rate, FCR = Feed conversion ratio.

Mean \pm SD in the same row with different superscript letters are significantly different ($P < 0.05$).

For the initial body weight and FCR the superscript denotes the ranking from highest to lowest.

differences observed in fish growth performance could be attributed to culture conditions, feed used and the respective aquaculture systems (Krishna et al., 2014; Shubhadeep et al., 2016). Further analysis also demonstrated that the growth of crossbred was better than the purebred cohorts. The positive heterosis of the body weight (3.8 %) provides valuable information to enhance the growth traits in the population. This finding suggests that genetic diversity and variation among the population of sea bass used in the study could be exploited for future genetic improvement programmes.

The survival rates of all cohorts were in the range of 99.5–100 %. Cohort 4 (Bangkok × Bali), cohort 6 (Bali × Malaysia) and cohort 8 (Malaysia × Bangkok) recorded the highest survival rate of 100 %, while cohort 5 (Bangkok × Malaysia) and cohort 7 (Bali × Bangkok) demonstrated the lowest survival rate of 99.5 %.

In terms of SGR, cohort 4 (Bangkok × Bali), cohort 6 (Bali × Malaysia) and cohort 8 (Malaysia × Bangkok) were significantly better compared to other cohorts within 2 months of the rearing period. There was a close relationship between SGR and fish weight, with SGR increasing as fish weight increases, as suggested by Jobling (1995).

The FCR documented in this study ranged from 1.2 to 1.5 and was in the range expected for carnivorous fish fed with artificial diets (Biswas et al., 2010; Phromkunthong et al., 2013). Cohort 4 (Bangkok × Bali) and cohort 6 (Bali × Malaysia) recorded the lowest FCR (1.2). Overall, cohort 4 (Bangkok × Bali) and cohort 6 (Bali × Malaysia) were the best-performing fish in terms of growth, FCR, and survival in tank culture.

Water Quality

Throughout quarantine, rearing and spawning of P₁ and F₁, physical water quality parameters such as dissolved oxygen, temperature, pH and salinity were monitored daily. Meanwhile, ammonia and nitrite were monitored every 5 days. The tanks were cleaned and syphoned daily by changing 80–100 % of the water. The tanks were refilled carefully with seawater to avoid stressing the fish. The water quality parameters during the rearing period did not differ significantly from one experiment to the other or between the different tanks. They were in the optimal range for Asian sea bass growth. The dissolved oxygen, temperature, pH and salinity recorded in the tanks were in the range of 4.12 ± 0.01 to 4.76 ± 0.01 mg.L⁻¹, 28.24 ± 1.12 to 29.26 ± 0.04 °C, 8.15 ± 0.05 to 8.32 ± 0.15, and 30.26 ± 0.40 to 30.33 ± 0.50 ppt, respectively. These readings of environmental parameters monitored in the tanks were within the range suggested by Kungvankij et al. (1986); Cheong (1989) and Schipp et al. (2007). The growth rate of cultured Asian sea bass that showed a substantial increase could be attributed to the favourable environmental parameters recorded.

After 5 years of implementation, this programme successfully produced eight cohorts despite the intended target of nine cohorts. To date, 503 pieces of the P₁ have been successfully distributed to five private hatcheries in Terengganu, Kelantan and Pahang, while 7,113 of the F₁ breeders with an average size 2.0–3.0 kg are ready to be selected as potential candidates in the production of F₂ generation. This programme will be continued in the 12th Malaysian Plan covering the period 2021–2025, with further improvement, as discussed in the next section.

Issues and Challenges

Although there are some encouraging achievements in this research and development, the broodstock development programme at FRI TD faced several significant challenges:

Lack of sufficient number of broodstock

The major problem is the difficulty in obtaining a sufficient number with a large genetic and phenotypic variation of broodstock. Since the number of hatcheries that produce broodstock in Malaysia is limited, imported broodstock is the main option. However, this option is also not fully dependable because imported broodstock were normally developed and adapted for use in a specific country. The imported broodstock may not display its full potential in different geographical locations. In addition, certain broodstock-producing countries also had exclusivity policies and were not aggressively promoting and expanding the resources to neighbouring countries.

In addition, a significant hindrance is achieving synchronised spawning of mating pairs to produce many families within a reasonable time interval (2–3 weeks). Farmed Asian sea bass exhibit precocious sex inversion before 2 years of age, and this phenomenon is the major impediment to maintaining broodstock in hatcheries. The broodstock generally reached maturity within two years of age in this programme. However, the sex ratio was uneven, with a much larger proportion of males than females at this stage. Furthermore, males after harvest (average 2.0 kg) were not ready to spawn, and the breeding failure rate was high. In both P₁ and F₁, parental fish were allowed to spawn naturally without any hormone injection.

High operational cost

The development of broodstock is a slow and high-risk process hence making the programme very costly. The execution of this project requires a high budget input for the development, operation and maintenance of the hatchery, which cannot be compromised. The stringent screening of pathogens at major steps of the programme also requires a significant budget. Therefore, this programme is

funded by the government. The investment in an effective breeding programme will only give high long-term economic returns because genetic improvement and continuous selection are cumulative over generations.

Inadequate infrastructure

The availability of a sufficient number of tanks and cages is crucial to maintaining the broodstock in fish breeding programmes especially marine fish, since the age at maturity is more than 2 years. At FRI TD, there are limited numbers of rearing tanks to accommodate all cohorts produced for performance tests in tanks. Consequently, the number of each cohort expected to produce in each generation (more than four) was not always achieved. Furthermore, despite the limited resources, FRI TD, also has an ongoing broodstock development programme on Grouper *Epinephelus* spp.

As a solution to this challenge, mass spawning and parentage assignment techniques using DNA markers to enable early communal rearing of all families may be an option for future breeding programmes for Asian sea bass. Another solution is by using cryopreservation techniques. Cryopreservation of sperm has been used as a strategy for conserving fish populations. Unfortunately, the cryopreservation of fish sperm, the establishment of a gene bank of Asian sea bass milt, and the integration of cryopreserved sperm use in hatchery practice are still in their early phase. The possible reasons why sperm cryopreservation is not widespread are that there are perceptions that the methods are too complicated, expensive or not reliable (Judycka et al., 2019).

Support and extension services

Besides the issues and challenges faced executing the broodstock development programme, there are also problems disseminating the potential broodstock to hatchery operators. Currently, seed dissemination is disorganised and needs a significant revamp. It is difficult to find reliable private hatcheries to accept the improved broodstock. Researchers have struggled to convince the industry regarding the benefits of selective breeding, and those expanding knowledge to aquaculture are unaware of its potential.

Future Perspective

There are prospects for expanding Asian sea bass culture in Malaysia due to its favourable production traits. The characteristics this species possess, e.g. hardiness, fast growth and suitability for culture in a wide range of environments, make it a preferred aquaculture species. This species' breeding, nursing and grow-out technology have been well established in Malaysia and provide ample commercial production opportunities. In addition, the large available potential

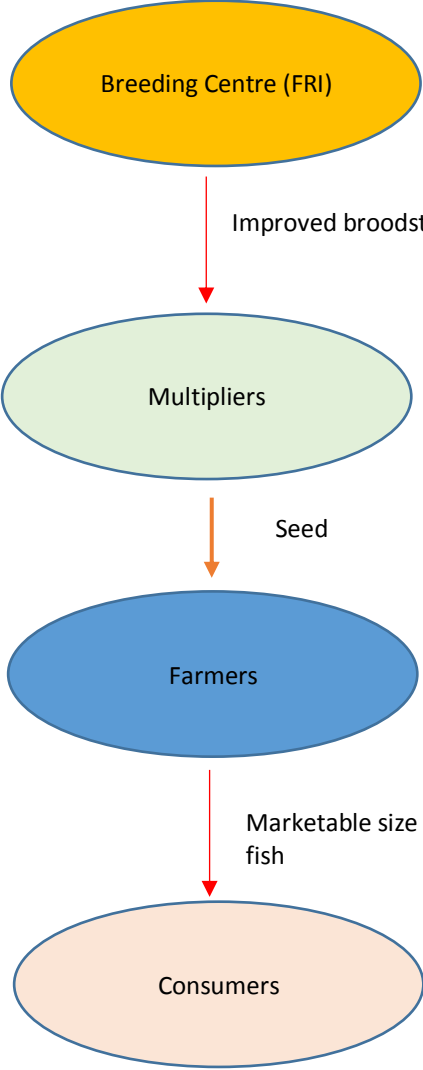
area for aquaculture in the country and the high market demand for this species accounts for a significant proportion of production in the future. FRI provides technical support from governments to improve broodstock quality, mass seed production, hatchery technology, feed formulation, grow-out and disease management. Furthermore, FRI will also transfer technology to hatchery operators and fish farmers to promote aquaculture production of Asian sea bass in Malaysia. Moreover, the financial support offered by the government and agriculture banks to potential entrepreneurs in this industry has also led to the sustainable development of aquaculture.

As the hatchery production and grow-out technology of Asian sea bass are well understood, the broodstock development programme is a practical solution to the lack of improved domesticated strain. This programme is in its early stages and has a long way to go. Further study should be conducted to understand the genetic survival parameters of sea bass, as cannibalism has caused a low survival rate at the nursery stage. In addition, studies on disease infection should be recorded to plan for a broader scope of research to produce a disease-resistant strain. Selection for a feed efficient strain is another area to be explored. Perhaps, a productive strain with high growth performance traits that require minimum feed during the culture period could be produced after several generations. Despite improving growth, the effect of genotype by environment interaction ($G \times E$) on this trait must be examined. Pham et al. (2018) reported $G \times E$ effects on body traits of Asian sea bass cultured in two different environments and when the body weight was measured at different ages of rearing periods. Thus, this finding suggests that further studies are required to determine an appropriate selection environment (ponds or cages) for the breeding programme.

Once the selection line is produced, the broodstock multiplier centre (BMC) of the improved bred has to be identified. This BMC is responsible for producing and distributing Asian sea bass seed to the farmers. To ensure the quality of the seed, this centre has to be accredited under Malaysian good aquaculture practice standard (MyGAP) and trained to follow strict broodstock management protocol. Therefore, sustained production of good quality seed could be achieved and disseminated. FRI must work with the government and private hatcheries as multipliers to disseminate the improved broodstock. This approach will ensure that the improved quality seed is available to fish farmers, benefiting from this programme.

Table 7 suggests the roles and activities of the Breeding Centre (FRI), the DOF, multipliers and fish farmers in ensuring the success of the Asian sea bass broodstock improvement programme in Malaysia. In brief, the genetic improvement programme takes place at the FRI where a base population is established and selection for the targeted traits is

Table 7. The roles and activities of Breeding Centre, Fisheries Research Institute (FRI), multipliers and fish farmers in Asian sea bass, *Lates calcarifer*, broodstock improvement programme in Malaysia.

Role	Activity
 <pre> graph TD BC([Breeding Centre (FRI)]) -- Improved broodstock --> M([Multipliers]) M -- Seed --> F([Farmers]) F -- Marketable size fish --> C([Consumers]) </pre>	<ol style="list-style-type: none"> 1. Establish base population 2. Family production 3. Performance assessment 4. Selection of broodstock 5. Broodstock dissemination <ol style="list-style-type: none"> 1. Identify and select hatcheries as 'Multiplier Centre' 2. Seed production and quality control 3. Seed distribution <p>Grow-out (cage and pond systems)</p>

made. This centre is commonly set up with government initiatives which incurs high investment and maintenance costs. When the improved strain is produced, the broodstock will be disseminated to selected hatcheries for multiplication. The selected hatcheries will mass-produce the seed from the improved broodstock and sell it to fish farmers. The hatcheries comply with certain standards to ensure quality seed is produced and distributed to fish farmers. Once the reproduction efficiency has decreased, the broodstock will be replaced by FRI. To avoid mating of close relatives, a sufficient number of broodstock from different sets of cohorts will be supplied to the hatcheries. Based on these roles, positive impacts of this programme will be perceived at farm and consumer levels.

Conclusion

The systematic breeding program of Asian sea bass, *Lates calcarifer*, in Malaysia has entered its 7th year of

implementation. The selected broodstock from Malaysia, Bali and Bangkok are used as base population. The program has produced until F₁ generation. The information on genetic variation and growth performance of P₀, P₁ and F₁ has been collected and analysed. Results obtained so far provide a tangible basis for growth improvement of Asian sea bass in Malaysia. Growth performance of F₁ has improved by more than 60 % in terms of weight gain compared to the base population. The improvement of growth indicates ample opportunities for genetic improvement for the next generations of sea bass. The genetic diversity among founder stocks could be exploited to enhance the growth traits. Furthermore, the positive heterosis of body weight indicated that the trait could be increased through selective breeding. Further development will likely focus on the development of F₂, adaptation and G × E effect, which is important for efficient breeding of stocks. The expansion of breeding objective to include improved disease resistance and reduced

cannibalism could be looked into maximise productivity and profit from Asia sea bass aquaculture.

Acknowledgements

This study was funded by the 11th Malaysian 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 to 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: Shaharah Md Idris: Planning, heading, executing the project and writing the manuscript. Wan Norhana Md Noordin: Drafting and writing the manuscript. Fatin Afifah: Assisting in the execution of the project, data analysis and writing. Azhar Hamzah: Planning, monitoring of the project, genetic data analysis, writing the manuscript.

References

- Ali, A. 1987a. Sea bass (*Lates calcarifer*) larvae and fry production in Malaysia. In: ACIAR Proceedings, No. 20, (eds. Copland, J.W., Grey, D.L.), pp. 144–147. Australian Centre for International Agricultural Research, Canberra.
- Ali, A. 1987b. Sea bass (*Lates calcarifer*) larvae and fry production in Malaysia. In: ACIAR Proceedings, No. 20, (eds. Copland, J.W., Grey, D.L.), pp. 165–167. Australian Centre for International Agricultural Research, Canberra.
- Ali, H.M. 1987c. Sea bass (*Lates calcarifer*) spawning in tanks in Malaysia. In: ACIAR Proceedings, No. 20, (eds. Copland, J.W., Grey, D.L.), pp. 129–131. Australian Centre for International Agricultural Research, Canberra.
- Aripin, A., Cogan, L., Pascoe, S., Viet, N.H. 2019. Productive efficiency and capacity utilization of sea bass grow-out culture in peninsular Malaysia. *Aquaculture Economics & Management* 24:101–121. <https://doi.org/10.1080/13657305.2019.1661045>
- Biswas, G., Thirunavukkarasu, A.R., Sundaray, J.K., Kailasam, M. 2010. Optimization of feeding frequency of Asian seabass (*Lates calcarifer*) fry reared in net cages under brackishwater environment. *Aquaculture* 305:26–31. <https://doi.org/10.1016/j.aquaculture.2010.04.002>
- Buendia, R. 1997. Sea bass grow-out and marketing: lessons from Australia, Malaysia, and Thailand. *SEAFDEC Asian Aquaculture* 19:27–28. <http://hdl.handle.net/10862/2916>
- Cheong, L. 1989. Status of knowledge on farming of sea bass (*Lates calcarifer*) in Southeast Asia. *Actes de colloques Ifremer, Tahiti, French Polynesia*, 20 Feb–4 Mar 1989, no. 9, chap. 39, pp.421–428. <https://archimer.ifremer.fr/doc/00000/1467/>
- Chu, K.B., Rashid, N.M., Rani, N.R.A. 2012. Infestation of gill copepod *Lernanthropus latis* (Copepoda: Lernanthropidae) and its effect on cage-cultured Asian sea bass, *Lates calcarifer*. *Tropical Biomedicine* 29:443–450.
- Department of Fisheries Malaysia. 2019. Annual fisheries statistics 2019. Department of Fisheries Malaysia. <https://www.dof.gov.my/index.php/pages/view/4046> (Accessed 05 February 2021).
- Dunstan, D.I. 1962. The barramundi in New Guinea waters. *Papua New Guinea Agriculture Journal* 15:23–31.
- Gjerde, B., Terjesen, B.F., Barr, Y., Lein I., Thorland, I. 2004. Genetic variation for juvenile growth and survival in Atlantic cod (*Gadus morhua*). *Aquaculture* 236:167–177. <https://doi.org/10.1016/j.aquaculture.2004.03.004>
- Husin, M.A., Ali, A. 1996. Aquaculture of coral reef fishes in Peninsular Malaysia. In: Workshop on aquaculture of coral reef fishes and sustainable reef fishes, Kota Kinabalu, Sabah. 14 pp.
- Hussin, M.A. 1987. Sea bass (*Lates calcarifer*) cage culture research in Malaysia. In: ACIAR Proceedings, No. 20, (eds. Copland, J.W., Grey, D.L.), pp. 69–71. Australian Centre for International Agricultural Research, Canberra.
- Jobling, M. 1995. Fish bioenergetics: Oceanographic Literature Review. 785 pp.
- Joerakate, W., Yenmak, S., Senanan, W., Tunkijanukij, S., Koonawootrittriron, S., Poompuang, S. 2018. Growth performance and genetic diversity in four strains of Asian sea bass, *Lates calcarifer* (Bloch, 1790) cultivated in Thailand. *Agriculture and Natural Resources* 52:93–98. <https://doi.org/10.1016/j.anres.2018.05.015>
- Judycka, S., Nynca, J., Ciereszko, A. 2019. Opportunities and challenges related to the implementation of sperm cryopreservation into breeding of salmonid fishes. *Theriogenology* 132:12–21. <https://doi.org/10.1016/j.theriogenology.2019.03.022>
- Katersky, R.S., Carter, C.G. 2005. Growth efficiency of juvenile barramundi, *Lates calcarifer*, at high temperatures. *Aquaculture* 250:775–780. <https://doi.org/10.1016/j.aquaculture.2005.05.008>
- Kechik, I.A. 1995. Towards sustainable aquaculture in Southeast Asia and Japan aquaculture in Malaysia. In: Proceedings of the seminar-workshop on aquaculture development in Southeast Asia, Iloilo City, Philippines (eds. Bagarinao, T.U., Flores, E.E.C.), pp. 125–135. Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines.
- Keenan, C.P. 2000. Should we allow human-induced migration of the Indo-West Pacific fish, barramundi *Lates calcarifer* (Bloch) within Australia? *Aquaculture Research* 31:121–131. <https://doi.org/10.1046/j.1365-2109.2000.00442.x>
- Krishna, S., Thirunavukkarasu, A.R., Kailasam, M., Prem, K., Subburaj, R., Thiagarajan, G. 2014. Pre-growout culture of Asian sea bass (*Lates calcarifer*) (Bloch) in low volume cage in brackishwater Ashtamudi lake under participatory mode with traditional fishermen. *Aquatic Biology and Fisheries* 2:272–276.
- Kua, B.C. 2008. The internal transcribed spacer (ribosomal DNA) of fish protozoan, *Cryptocaryon irritans*, isolated from cultured sea bass, *Lates calcarifer*, in Penang waters. *Asian Fisheries Science* 21:285–292.
- Kungvankij, P., Pudadera, B.J. Jr., Tiro, L.B. Jr., Potestas, I.O. 1986. Biology and culture of sea bass (*Lates calcarifer*). UNDP/FAO, NACA Training Manual Series, No. 3. NACA, Bangkok. 70 pp.
- Liong, P.C., Hanafi, H.B., Merican, Z.O., Nagaraj, G. 1988. Aquaculture development in Malaysia. In Proceedings of the seminar on aquaculture development in Southeast Asia, Iloilo City, Philippines (eds. Juario, J.V., Benitez, L.V.), pp. 73–90. Aquaculture Development in Southeast Asia and Japan: Contributions of the SEAFDEC Aquaculture Department, Philippines.
- Macbeth, G.M., Palmer, P.J. 2011. A novel breeding programme for improved growth in Barramundi, *Lates calcarifer* (Bloch) using foundation stock from progeny-tested parents. *Aquaculture* 318:325–334. <https://doi.org/10.1016/j.aquaculture.2011.05.037>
- Mackinnon, M. 1989. Status and potential of Australian *Lates calcarifer* culture. *Advances in Tropical Aquaculture* 65:713–727. *Actes de*

- colloques Ifremer, Tahiti, French Polynesia. <https://archimer.ifremer.fr/doc/00000/1429/>
- Muhammad, M.Z. 1993. Seed production of marine fish in Malaysia. In: Proceedings of the aquaculture workshop for SEAFDEC/AQD training alumni, Iloilo, Philippines, pp. 21-26. SEAFDEC Aquaculture Department, Philippines.
- Nguyen, N.H. 2016. Genetic improvement for important farmed aquaculture species with a reference to carp, tilapia and prawns in Asia: Achievements, lessons and challenges. *Fish and Fisheries* 17:483-506. <https://doi.org/10.1111/faf.12122>
- Norfatimah, M.Y., Siti Azizah, M.N., Othman, A.S., Patimah, I., Jamsari, A.F.J. 2009. Genetic variation of *Lates calcarifer* in Peninsular Malaysia based on the cytochrome b gene. *Aquaculture Research* 40:1742-1749. <https://doi.org/10.1111/j.1365-2109.2009.02279.x>
- Pham, V.K. 2019. Improving seed production and genetic improvement of Asian sea bass *Lates calcarifer*. https://research.usc.edu.au/discovery/fulldisplay/alma99451210202621/61USC_INST:ResearchRepository (Accessed 15 February 2021).
- Pham, V.K., Truong, H.P., Nguyen, K.D., Wayne, K., Nguyen, H. 2018. An 8-Year breeding program for Asian sea bass *Lates calcarifer*: Genetic evaluation, experiences, and challenges. *Frontiers in Genetics* 9:191. <https://doi.org/10.3389/fgene.2018.00191>
- Phromkunthong, V., Nuntapong, N., Boonyaratpalin, M., Kiron, V. 2013. Toxicity of melamine, an adulterant in fish feeds: Experimental assessment of its effects on tilapia. *Journal of Fish Diseases* 36:555-568. <https://doi.org/10.1111/jfd.12003>
- Ransangan, J., Lal, T.M., Al-harbi, A.H. 2012. Characterization and experimental infection of *Vibrio harveyi* isolated from diseased Asian sea bass (*Lates calcarifer*). *Malaysian Journal of Microbiology* 8:104-115. <https://doi.org/10.21161/mjm.03512>
- Ransangan, J., Manin, B.O., Abdullah, A., Roli, Z., Sharudin, E.F. 2011. Betanodavirus infection in Golden Pompano, *Trachinotus blochii*, fingerlings cultured in deep-sea cage culture facility in Langkawi, Malaysia. *Aquaculture* 315:327-334. <https://doi.org/10.1016/j.aquaculture.2011.02.040>
- Sakaras, W. 1987. Optimum stocking density of sea bass (*Lates calcarifer*) cultured in cages. In: ACIAR proceedings of an international workshop (eds. Copland, J.W., Grey, D.L.), pp. 172-178. Darwin, N.T. Australia.
- Schipp, G., Jerome, B., John, H. 2007. Barramundi farming handbook. Department of Primary Industry, Fisheries and Mines, Darwin Aquaculture Centre, Darwin NT. 80 pp.
- Shubhadeep, G., Sekar, M., Ritesh, R., Biswajit, D., Phalguni, P., Loveeson, E., Biju, X. 2016. Growth performance of Asian sea bass (*Lates calcarifer*) (Bloch 1790) stocked at varying densities in floating cages in Godavary Estuary, Andhra Pradesh, India. *Indian Journal of Fisheries* 63:146-149. <https://doi.org/10.21077/ijf.2016.63.3.49095-22>
- Szekely, C., Borkhanuddin, M.H., Shaharom, F., Embong, M.S.A., Molnar, K. 2013. Description of *Goussia kuehae* n. sp. (Apicomplexa: Eimeriidae) infecting the Asian seabass, *Lates calcarifer* (Bloch) (Perciformes: Latidae), cultured in Malaysian fish farms. *Systematic Parasitology* 86:293-299. <https://doi.org/10.1007/s11230-013-9448-1>
- Wang, C.M., Lo, L.C., Zhu, Z.Y. 2006. A genome scan for quantitative trait loci affecting growth-related traits in an F1 family of Asian sea bass (*Lates calcarifer*). *BMC Genomics* 7:274. <https://doi.org/10.1186/1471-2164-7-274>
- Weiss, S., Schlotterer, C., Waidbacher, H., Jungwirth, M. 2001. Haplotype (mtDNA) diversity of brown trout *Salmo trutta* in tributaries of the Australian Danube: Massive introgression of Atlantic basin fish by man or nature? *Molecular Ecology* 10:1241-1246. <https://doi.org/10.1046/j.1365-294X.2001.01261.x>
- World Bank. 2017. Driving performance from the center: Malaysia's experience with PEMANDU. World Bank, Washington, DC. 68 pp.
- Yue, G.H., Zhu, Z.Y., Lo, L.C., Wang, C.M., Lin, G., Feng, F., Pang, H.Y., Li, J., Gong, P., Liu, H.M., Tan, J., Chou, R., Lim, H., Orban, L. 2009. Genetic variation and population structure of Asian sea bass (*Lates calcarifer*) in the Asia-Pacific region. *Aquaculture* 293:22-28. <https://doi.org/10.1016/j.aquaculture.2009.03.053>