Fisheries Research Institute, Department of Fisheries Malaysia

FRI NEWSLETTER

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MESSAGE FROM *the Editor*

Welcome to the 30th-anniversary edition of the FRI Newsletter. We are proud and excited to bring the FRI Newsletter back into print for its 30th consecutive year. FRI Newsletter is published yearly to disseminate current findings, activities, and transfer-of technology conducted by the FRI. Initiated in 1994, FRI Newsletter is still committed to its founding principles of disseminating brief FRI R&D outputs to the reader. Initially, the contents of the FRI Newsletter were general; however, since 2018, we have come up with a specific theme for each year. From highlighting the FRI collaboration projects in 2017, broodstock developments R&D in 2018, aquafeed R&D in 2019, capture fisheries R&D in 2020, aquatic invasive species R&D in 2021, R&D at the South China Sea in 2022, and the latter R&D in ornamental fish and aquatic plants last year. The current issue of the FRI newsletter is dedicated to highlighting the progress and status of the R&D executed under the 12th Malaysia Plan.

If one thing has not changed since 1994, it is the fact that the FRI Newsletter serves to encourage researchers to write and share their findings and thoughts. I would like to invite all researchers to engage us with ideas, comments, or suggestions to improve this newsletter further. Please get in touch with me at norhana@dof.gov.my for your valuable input.

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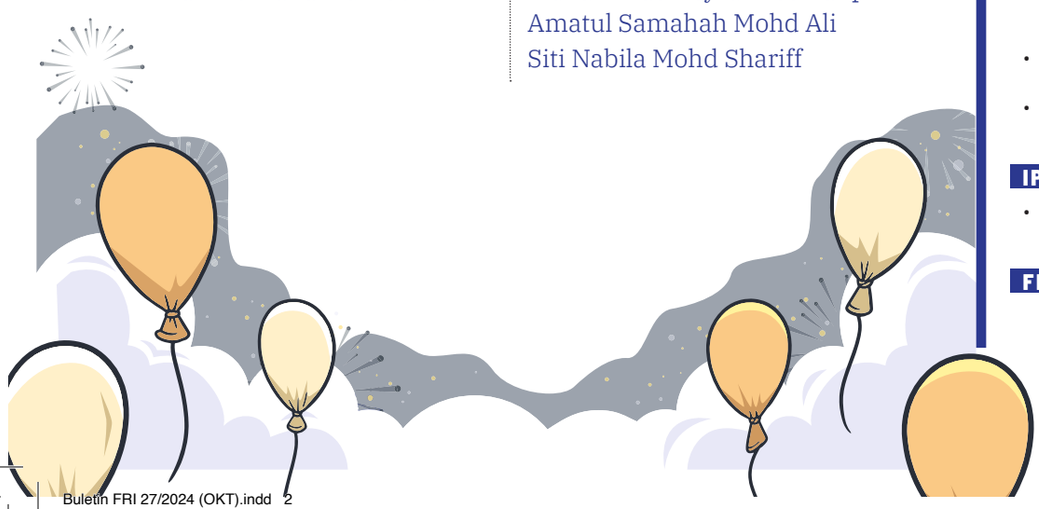

What's inside

Fisheries Research Institute
12th Malaysia Plan R&D Programmes at a Glance

WAN NORHANA MN* &
MUHAMMAD FAROUK H
Fisheries Research Institute,
11960, Batu Maung, Penang
norhana@dof.gov.my

Introduction

A five-year Malaysia Plan is a comprehensive outline of government development policies and strategies initiated since 1995. We are currently in the 12th Malaysia Plan (MP) (2021–2025) with the primary objective of achieving a prosperous, inclusive, and sustainable Malaysia. The 12th MP is anchored on three key themes and supported by four catalytic policy enablers (Fig. 1). R&D is entrusted with accelerating technology adoption and innovation policy enablers by aligning the R&D towards commercialization, wealth generation, and economic growth. This article provides pertinent information regarding the R&D projects at the Fisheries Research Institute in the 12th MP, the outputs achieved in RP1, RP2, and RP3, and the challenges faced.

Fund received

At the beginning of the 12th MP, RM 56.275 million was allocated to carry out R&D projects under two programmes in Peninsular Malaysia (RM 40 million) and four (RM 16.275 million) in Sarawak. Later, in RP3 and RP4, two more R&D programmes worth RM 4.24 million were approved. Additionally, FRI received two physical projects in RP3 specifically to upgrade facilities in FRI Pulau Sayak, Kedah (RM 7.5 million) and FRI Glami Lemi, Jelebu, Negeri Sembilan (RM 2.4 million). Up to January 2024, the FRI received RM 70.415 million, representing approximately 11.5% of the total allocation disbursed to the Department of Fisheries (DOF). Table 1 lists the details of the approved R&D allocation in the 12th MP according to each RP.

In the 11th MP, RM 63.55 million was received for R&D, a 16% reduction compared with the 12th MP. There is a major slash in the grant received for R&D in Aquaculture (P21300040170501) in 12th MP (RM 20 million) compared

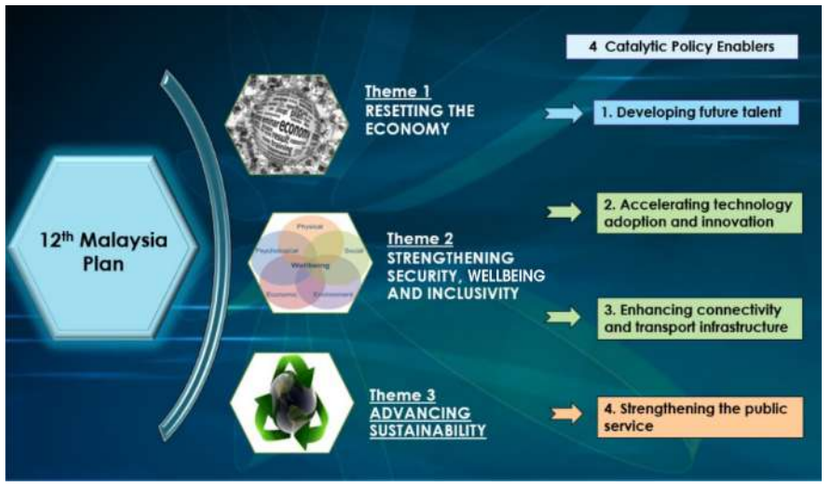

Figure 1: Three key themes and four policy enablers of the 12th Malaysia Plan

Table 1: Main R&D and C&I programmes approved in the 12th MP Plan and the budget received in each Rolling Plan (RP)

	Title	Amount Received (RM million)					
		RP1	RP2	RP3	RP4	RP5	Total
Non-Physical (R&D) programme	1. Research, Development, and Commercialization of Sustainable Aquaculture Technology (P21300040170501)	3.0	7.0	3.6	3.4	3.0	20.0
	2. Research and Development in Aquaculture Fish Health (P21300040170502)	1.6	5.0	5.0	5.0	3.4	20.0
	3. Oceanic Fisheries Resources and Bio socioeconomics of Capture Fisheries towards Integrated Success in the Whole State of Sarawak (P21300060132003)	1.0	0.8	1.4	0.8	-	4.0
	4. Strategic Research of Islands and Shoals in the South China Sea (P21300060132004).	0.5	1.0	0.5	0.5	0.25	2.75
	5. Development of New Methods in Improving the Survival and Health of Shrimp/Fish in Aquaculture in Sarawak (P21300060132005).	0.5	0.8	1.5	0.5	1.275	4.575
	6. Natural and Anthropogenic Impact Assessment Research on the Fishing Industry in Sarawak (P21300060132006).	0.3	1.0	1.4	2.99	-	5.69
	7. Impact Research and Risk of Pollution and Harmful Algae to the Fisheries Industry (P21300040170509).	Nil	Nil	1.0	1.17	1.07	3.24
	8. Survey of Marine Fish Resources: Application of New Assessment Methodologies in Ensuring Food Security (P21300040170512)	Nil	Nil	Nil	0.5	0.5	1.0

Note: RP-Rolling Plan

with 11th MP (RM 41.6 million), which is almost half. This slash is a major setback to this program as the fund was divided and shared between six FRIs with 25-30 researchers. However, there is a two-time surge in the amount of allocation received for Aquaculture Fish Health Research (P21300040170502) in the 12th MP (RM 20 million) compared to the 11th MP (RM 10.2 million), which only involved one center with only seven research officers. Meanwhile, the allocation for R&D on Capture Fisheries and Impact Assessment was only granted in RP3. Fortunately, R&D in these two areas could still be implemented with financial support from other Headquarters DOF divisions. The total expenditure in each RP is almost 100%.

Books published in 2021, 2022, 2023, and 2024

Main Output

Among the main outputs of R&D are the publication and dissemination of research findings, transfer of technology, and development of new technology, innovation, and intellectual properties (IP). The number of journal articles published in RP1, RP2, and RP3 are 14, 20, and 45, respectively. Although the number of journal publications increases in each RP, it is still low compared to the number of researchers and the amount of funds received. Besides journals, researchers also published books to disseminate their research findings (Fig. 1). The most common publication is 'grey literature,' which includes abstracts in annual reports, technical reports, and presentations at seminars, webinars, or conferences. As for IP registration, a total of 29 intellectual properties (IP) were registered in 2021 (2 utility innovations and 14 copyrights), 2022 (1 trademark, 2 industrial designs, and 8 copyrights), and 2023 (1 patent and 1 copyright,). The new IP registration (1 patent, 2 utility innovations, 1 trademark, and 4 copyrights) for 2024 is underway. Although the IP number seems to decline from the 11th MP, this is justifiable as most IP generation usually takes time. The technology started in the 12th MP and often required more than 5 years to test and evaluate it thoroughly before it could be filed.

The significant new technologies introduced in the 12th MP were the cost-effective farm-scale fish feed extruder machine (MSPEX), AI-based Dissolved Oxygen Detection System and Paddle Wheel Control in the Ponds, and IoT Automation System for Water Quality Detection Control in Tanks. These technologies are currently being validated by the industrial partners throughout the Peninsular Malaysia. MSPEX won the gold medal (public service category) and a special award from the Chinese Innovation and Invention Society, Taiwan, during the Malaysia Technology Expo (MTE) 2022. Meanwhile, R&D on cockles-induced spawning also achieved significant highlights in the 12th MP. Besides increasing the yearly income of the fisherman in Lekir, Perak, the R&D also gained a gold medal (public service category) and a special award by the National Research Council of Thailand for the innovation of cockles-induced spawning in the MTE 2023. In 2024, six FRI innovations bagged gold medals in the International Invention, Innovation & Technology Exhibition (ITEX) 2024, Kuala Lumpur. Along with this, a significant number of technology transfer and advisory services in aquaculture and fisheries management were executed throughout RP1–RP4 of 12th MP.

Issues and Challenges

The foremost obstacle in implementing R&D in the 12th MP and other MPs is the budget shortage for travel and transportation (T&T). Almost all R&D projects require researchers to travel by land or sea to the study locations, which involves high expenditures. Besides the downsizing of research staff, FRI needs to rely on contract staff to assist in executing R&D. The current inflexibility with the scope of the budget regarding these two crucial circumstances has caused significant glitches in the project implementation. This matter was further aggravated by the lower T&T allocation received yearly.

Conclusion

The 12th MP is already slightly more than halfway through its implementation. The FRI reviewed most projects during the Fisheries Research Seminar (12th MP Mid Term Review), 7–9 March 2023, Penang. It is hoped that the project leaders and managers could take the necessary steps to rectify all the shortcomings, expedite the project implementation, and achieve all the outputs as promised in the project submission. ■

Enzyme-linked Immunosorbent Assay (ELISA) for **Viral Nervous Necrosis Detection in Asian Seabass (*Lates calcarifer*)** using Cell Culture Product as Coating Antigen

AZILA A[1]*, IRFAN HAKIMI R[2] & NUR NAZIFAH M[2]

[1] National Fish Health Research Centre (NaFisH), 11960 Batu Maung, Pulau Pinang
[2] Kulliyah of Science, International Islamic University Malaysia, Bandar Indera Mahkota, 25200 Kuantan, Pahang,
*azila@dof.gov.my

Introduction

Betanodavirus infection, commonly known as viral nervous necrosis (VNN), is brought on by the nervous necrosis virus (NNV). VNN primarily attacks the brain and eye regions of the fish nervous system and is also referred to as viral encephalopathy and retinopathy (VER). It is an icosahedral-shaped, non-enveloped, single-stranded, positive sense RNA virus with a diameter of 25–30 nm. Its significant implication is that it causes 100% mortality in fingerlings and flies as early as 10 days old (Ariff et al., 2019) because of the virus's vertical transmission features.

Numerous techniques have been developed for identifying VNN in fish, ranging from molecular to cell culture techniques and immunoassay detection. Since 2006, NaFisH has developed and used reverse transcriptase polymerase chain reaction (RT-PCR) and, later, real-time polymerase chain reaction (qPCR). VNN is currently considered endemic to Malaysia and may be present in "healthy-looking" fish anywhere in the country. Therefore, from an immunological perspective, our marine fish are presumed to possess already a "certain" quantity of natural antibodies that protect them from this disease; nevertheless, further research is necessary to substantiate this with sound scientific data.

Enzyme-linked immunosorbent assay (ELISA), a widely used biochemical method, is used to determine whether a sample contains particular proteins, antibodies, or antigens. It is a quantitative and sensitive approach with applications in environmental monitoring, research, and clinical diagnostics. ELISA is essential to the aquaculture sector as it can support research & development of vaccines, health monitoring, and disease diagnosis. It is a crucial instrument in guaranteeing the sustainability and productivity of aquaculture operations because of its sensitivity, specificity, and versatility in determining the infection status of the disease. Considering the aforementioned information, this research seeks to develop an ELISA method to improve the control and prevention strategy for VNN.

Methodology

(a) Development of Hyperimmune Serum

Hyperimmune Serum (HIS) was developed to obtain the specific enzyme-linked antibodies (Rabbit anti-seabass) required for detecting VNN in *Lates calcarifer*. For the development of HIS, serum of *L. calcarifer* was obtained from Fisheries Research Institute Tanjung Demong was emulsified with an equal volume of Freund's Complete Adjuvant (FCA) and injected subcutaneously into rabbits (Mori et al., 2003). Booster serum injections without FCA were administered twice (days 7 and 14). Blood was withdrawn from the marginal ear vein of a rabbit on day 28, and sera was stored at −20°C until further use.

(b) Development of ELISA method for VNN detection in Asian seabass

Preparation of coating antigen

ELISA was performed as recommended by Choi et al. (2012) and Kim et al. (2008), with minor adjustments to the coated antigen preparation. The ideal centrifugation period (20, 40, and 60 min), centrifuge force (10000, 20000, and 30000 g), and incubation temperature (4°C and 37°C) were established to thoroughly remove the cell debris from the virus culture (Fig. 1).

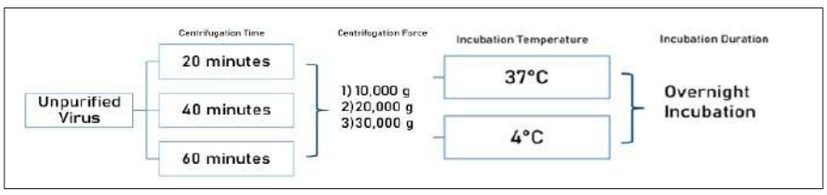

Figure 1: Determination of optimum centrifugation time, force, and temperature for preparing coating antigen

ELISA procedure

After overnight incubation, ELISA was carried out using the methodology shown in Fig. 2.

Ferreira et al. (2019) have stated that culture fluids of viruses were valuable as ELISA capture antigens for detecting specific antibodies in fish sera.

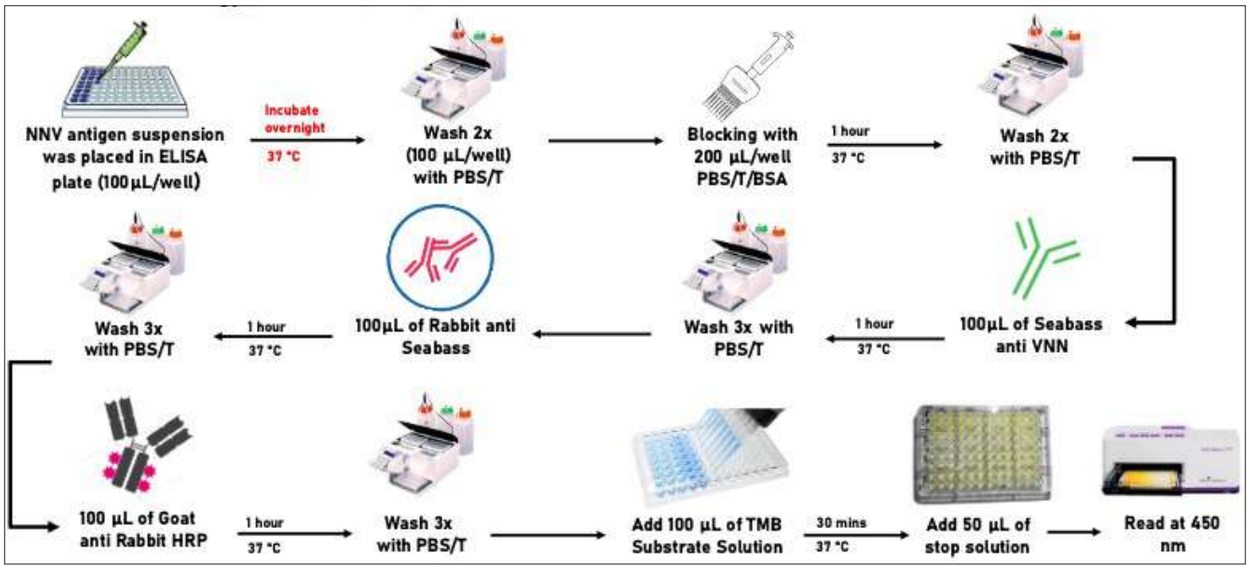

Figure 2: ELISA procedure for VNN detection in Asian seabass

(c) Determination of antibody dilution

Checkerboard experiments were conducted to find the ideal antibody dilution for the conventional procedure (Fig. 3). Throughout the process, three enzyme-linked antibodies were used: 1° Seabass anti-VNN, 2° Rabbit anti-Seabass, and 3° Goat anti-Rabbit HRP. Four distinct dilution concentrations were selected for the primary antibody, Seabass anti-VNN (1:200, 1:400, 1:600, and 1:800), and secondary antibody, Rabbit anti-Seabass (1:500, 1:1000, 1:1500, and 1:2000). As illustrated in Fig. 2, checkerboard was performed using the serum of Asian seabass, both infected and healthy, and the ideal dilution was established by reference to a work conducted by Kim et al. (2008).

Checkerboard Results (Positive Control Group)

SeVNN / Rbs	1:200	1:400	1:600	1:800
1:500	0.317	0.337	0.295	0.333
1:1000	0.217	0.236	0.256	0.258
1:1500	0.187	0.228	0.260	0.257
1:2000	0.231	0.246	0.241	0.241

Checkerboard Results (Negative Control (Healthy) Group)

S (Healthy) / RbT	1:200	1:400	1:600	1:800
1:500	0.089	0.087	0.086	0.071
1:1000	0.085	0.084	0.079	0.065
1:1500	0.082	0.081	0.075	0.063
1:2000	0.079	0.074	0.070	0.060

Figure 3: OD value for the positive control group and negative control group from the checkerboard

Results and Discussion

Based on the results from Fig. 3, the optimum dilutions for the standard method were 1:800 and 1:1500 for the primary antibody (seabass anti-VNN) and secondary antibody (Rabbit anti-seabass), respectively. Dilutions of 1:800 for the primary antibody and 1:1500 for the secondary antibody were enough to achieve the OD values within the positive control group (0.26–1.03) (Kim et al., 2008). This method, using virus culture products as antigens, made it possible to detect specific antibodies in fish sera. Breuil & Romestand (1999) and Soon, a quick investigation will be planned to gather information on the natural antibody defence in our various marine-cultured fish. This technique should help establish more effective VNN control and preventive strategies. Adding this new ELISA approach to the group of existing VNN detection methods created during the 9th to 12th MP will lead to more thorough and intriguing findings in VNN research in the future.

Conclusion

This research study developed an effective, efficient, easy, and flexible ELISA procedure for VNN detection in Asian seabass. An advantage of this method is the replacement of purified virus with virus culture fluid as a coating antigen, which is much easier, cheaper, and time-saving. ■

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Marine Habitat Mapping of Northern Langkawi, Kedah

MD. NIZAM I*, ZAIDNUDDIN I & MS ISMAIL
Fisheries Research Institute, 11960 Batu Maung, Penang
*mdnizam@dof.gov.my

Langkawi is located at the Northern Straits of Malacca (NSOM), adjacent to the Andaman Sea, the North-eastern Indian Ocean water body. It is adjacent to the UNESCO Satun Global Geopark in Thailand. The Andaman Sea, including the NSOM, is an important area for marine biodiversity and a significant part of the Indian Ocean, with its unique ecosystem and marine life.

Marine habitats are essential for various reasons, including their importance for marine life, the health of marine ecosystems, and their impact on human livelihoods. Marine habitats can be protected by establishing Marine Protected Areas (MPAs), implementing responsible fishing practices, and reducing pollution in marine environments. Mapping marine habitats will become more important if conservation efforts between Malaysia and Thailand through the Satun-Langkawi Archipelago UNESCO Global Geopark come to fruition. The output of this project may provide scientific evidence, especially on the distribution of coral reef areas, to support northern Langkawi as part of transboundary MPAs. The habitat mapping project conducted consists of nine sites in the north part of Langkawi: Teluk Tanjung Tembun, Teluk Datai, Teluk Sungai Langgara, Pulau Jemuruk, Teluk Yu, Pulau Dangli, Pulau Gasing, Pulau Pasir, and Teluk Pasir Panjang (Fig. 1). Coral reefs here are very vulnerable to possible drastic climate change because of their connection with the Andaman Sea.

The method used for habitat mapping was the Acoustic Ground Discrimination System (AGDS) *RoxAnn*, which was attached to the boat with GPS and towed at an average speed of 3 to 4 knots (Fig. 2). The boat scanned the seabed with a "U"-shaped transect, and the distance of the transect was between 5 m and 10 m. The captured data, consisting of depth and seabed type, was analysed with Surfer V.13.0 for thematic maps.

The classification of seabed type discriminates the habitat into five categories: live coral cover (LCC), dead coral with algae, sand, silt, and rock. The highest LCC was recorded at Teluk Datai, with an area of 38,896 m^2 (3.05%), followed by Teluk Pasir Panjang (10,983 m^2 @ 2.93%), Teluk Pasir Panjang (10,983 m^2), Teluk Tanjung Tembun (10,821 m^2 @ 4.98%), Pulau Pasir (10,456.99 m^2 @ 6.31%), Pulau Jemuruk (7,993 m^2), Pulau Dangli (7,193 m^2), Pulau Gasing (6,665 m^2), and Teluk Sg. Langgora (2,292 m^2) (Fig. 3). The most dominant habitat type was sand (73%), followed by silt (16%), dead coral with algae (6%), coral (4%), and lastly rock (1%).

The Northern Langkawi marine habitat also supports the presence of sea turtles such as hawksbill turtles

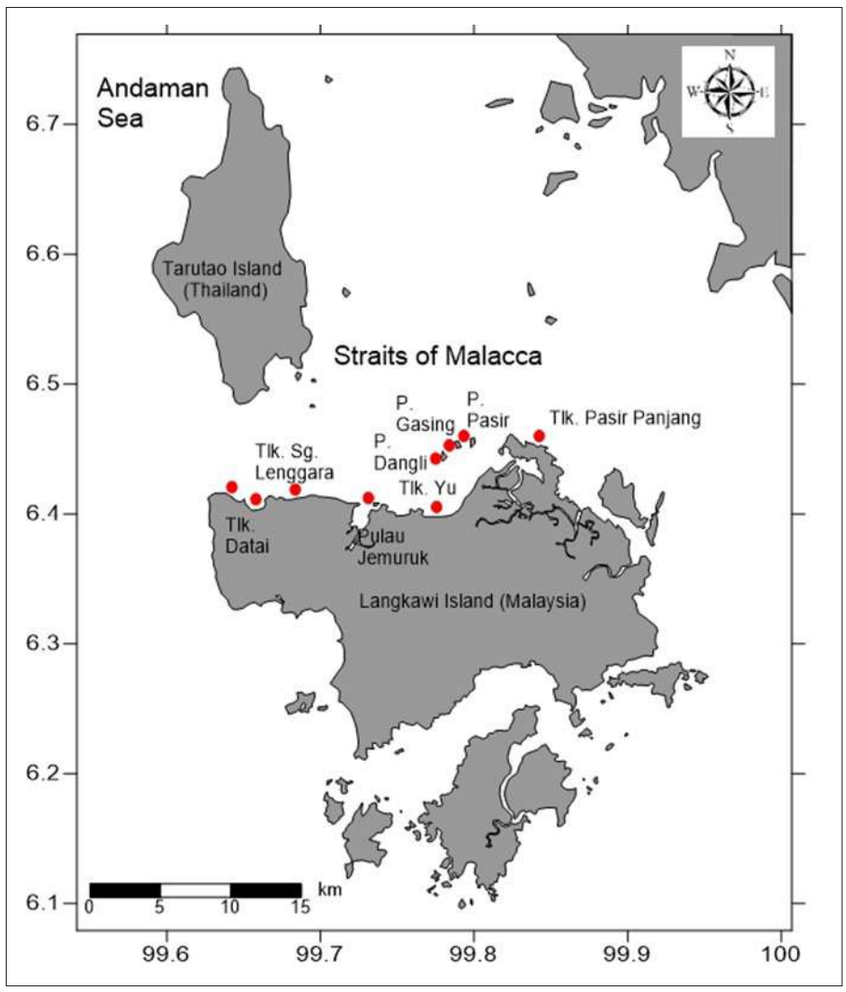


Figure 1: Study sites

The Northern Langkawi marine habitat also supports the presence of sea turtles such as hawksbill turtles (*Eretmochelys imbricata*) and green turtles (*Chelonia mydas).*

Figure 2: Illustration of data collection

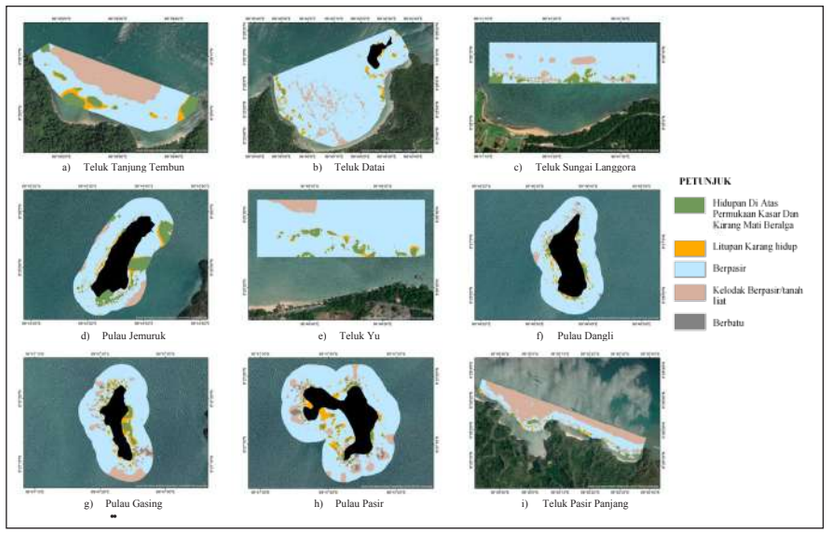
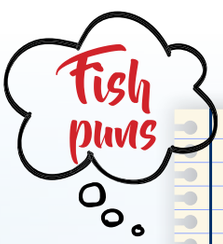


Figure 3: Map of habitat type in the Northern Langkawi

(*Eretmochelys imbricata*) and green turtles (*Chelonia mydas).* Marine mammals include Bryde's whale *(Balaenoptera edeni),* Indo-Pacific finless porpoise, Indo-Pacific humpback dolphin, and whale shark (*Rhincodon typus*).

From this study, habitat types in the Northern Langkawi were various and could significantly contribute to the survivability of the coral reef, with the important element being MPAs. Nevertheless, other research disciplines, such as socio-economics and physico-biology, must be blended to get a clearer picture of the potential of this area to become the first transboundary MPA between Malaysia and Thailand. ■



WHY ARE FISH SO EASY TO WEIGH?
Because they have their own scales!

WHAT DO YOU CALL A FANCY FISH?
So-fish-ticated!

WHY DID THE FISH REFUSE TO SHARE ITS FOOD?
It was a little shellfish

WHAT KIND OF FISH ONLY COMES OUT AT NIGHT?
A starfish!

WHAT FISH PERFORMS OPERATIONS?
A sturgeon!

WHAT YOU CALL A RICH FISH?
A goldfish

WHAT IS IT VEGETABLE ON LAND BUT ANIMAL IN SEA?
A sea-cucumber

WHAT IS ANIMAL THAT MALE GIVE BIRTH?
A seahorse

MOHD LAZIM MS*, AZMI R, MOHD FARAZI J, FADZILAH Y, AZLINA A & NUR AMALINA MR
Fisheries Research Institute Gelang Patah, 81550 Gelang Patah, Johor
lazim_saif@dof.gov.my

Super-Intensive White Shrimp Culture: Promising Yield of 50 MT/hectares

The Department of Fisheries, Aquaculture Strategic Plan 2021–2030 focuses on the revolution of the aquaculture industry, especially marine shrimp farming, which is also in line with the National Agrofood Policy 2021–2030. The target production of marine shrimp aquaculture by 2030 is expected to reach 109,000 metric tons (MT) worth RM 2.1 billion. For the record, marine shrimp production in 2022 was 48,000 mt (DOF, 2023). To achieve this goal, the Fisheries Research Institute (FRI), Gelang Patah, has introduced a super-intensive white shrimp farming system, as it is almost impossible to achieve the target through conventional farming, even if a hundred acres of farming areas were involved. The R&D on super-intensive shrimp farming system development was initiated in the 11th Malaysia Plan (MP) and, at the end of the 11th MP, was able to produce shrimps up to 40 MT/hectares compared to the conventional method (10-15 MT/hectares). This system uses small ponds/tanks of 0.0005, 0.001, 0.01, and 0.1 hectares.

This project was continued in the 12th MP under the Research, Development, and Commercialization of Sustainable Aquaculture Technology Program (P21300040170501) with further improvement of the system and an increase in stocking density. The newly improvised system does not require a larger area; instead, it is characterized by smaller size, higher density, and systematic and environmentally friendly organic waste management. Table 1 presents the total production of shrimps with different stocking rates and pond/tank sizes.

FRI Gelang Patah has been working on super-intensive shrimp farming in 0.001 ha tanks and 0.1 ha high-density polyethylene tanks, with an initial stocking density of 300–400 Post Larvae/m^2. The yield obtained from this system reached up to 45.0 - 51.0 MT/ha/cycle without having a negative impact on the water quality and growth performance of shrimp. This output was better than the current commercial farm production.

However, super-intensive farming requires experienced and skilled operators to ensure their success. The key to the success of this super-intensive farming system technology is the ability to overcome problems that arise by focusing on elements of the farming system, livestock management, and water quality management. This high-density farming requires farmers to manage it efficiently, with knowledge and extensive experience in shrimp farming. The concept of partial harvest is practiced to allow the shrimp to grow well and reach the desired production target.

Pre-commercialization of this technology is actively being promoted. The states that have received and adopted this system include Johor, Pahang, Terengganu, and Selangor. FRI Gelang Patah researchers visited the farms, provided technical advice, and exchanged views on issues that arose during the farming period. ■

Table 1: Total production of shrimps with different stocking rates and pond/tank sizes

Variable	Stocking density (pieces/meter^2)					
	200		300		400	
Pond size (hectare)	0.01	0.1	0.01	0.1	0.01	0.1
Survival rate (%)	98	87.8	93	94.0	77	93
Food Conversion Ratio	1.68	1.48	1.23	1.30	1.31	1.5
Average final weight (g)	27.40	22.59	29.20	26.29	26.10	25.96
Growth rate (g/day)	0.23	0.17	0.24	0.26	0.22	0.22
Production (kg)	3,240	3,000	4,461	4,703	4,903	5,100
Production (metric tonnes/ha/cycle)	32.40	30.0	44.61	47.03	49.03	51.00

Is **Sungai Pulai, Johor,** Suitable **for Aquaculture Endeavour?**

NUR AMALINA MR*, FADZILAH Y & AZLINA A
Fisheries Research Institute Gelang Patah, 81550 Gelang Patah, Johor
*amalina@dof.gov.my

Sungai (Sg.) Pulai is one of the major river basins (>80 km^2) and the largest mangrove forest ecosystem in Johor. It was the first mangrove forest to be gazetted in 1923 and listed as a RAMSAR site for its productive habitat with high ecological and economic importance in 2003. Sg. Pulai flows from Gunung Pulai to Tanjung Pelepas and continues to the Tebrau Strait. In the past, Sg. Pulai has been the livelihood for fishermen around Gelang Patah, Pendas, and Skudai, Johor. This river is the primary water source for R&D activities at the Fisheries Research Institute Gelang Patah, and nearby fish/shrimp farming. Nevertheless, urbanization and industrialization have impacted the quality of the river water and the livelihood of the fishermen.

Thus, it is necessary to investigate the current water quality status of Sg. Pulai. This project was conducted under the 12th Malaysia Plan under Research, Development, and Commercialization of Sustainable Aquaculture Technology (P21300040170501). The project aims to determine major water quality parameters along Sg. Pulai.

Water quality parameters such as dissolved oxygen (DO), total suspended solids (TSS), and pH were measured *in situ*, whereas other analyses (biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia and nitrogen levels) were analysed in the lab. Water quality analyses were conducted at eight designated stations along the Sg. Pulai, covering upstream, downstream, and estuary areas (Fig. 1). The readings obtained were referred to the Water Quality Index (WQI) (Table 1) and classification by the Department of Environment Malaysia (DOE, 2020) (Table 2).

Figure 1: Sampling stations at Sungai Pulai, Johor

Table 1: Water quality classification based on the Water Quality Index Water quality classification by the Department of Environment Malaysia (DOE, 2020)

Sub Index & Water Quality Index	Index Range		
	Clean	Slightly Polluted	Polluted
Biochemical Oxygen Demand (BOD)	91–100	80–90	0–79
Ammoniacal Nitrogen (NH_3-N)	92–100	71–91	0–70
Suspended Solids (SS)	76–100	70–75	0–69
Water Quality Index (WQI)	81–100	60–80	0–59

Table 2: Water quality classification by uses by the Department of Environment

Class	Uses
Class I	Conservation of the natural environment Water Supply I – Practically no treatment necessary Fishery I – Very sensitive aquatic species
Class IIA	Water Supply II – Conventional treatment required. Fishery II – Sensitive aquatic species.
Class IIB	Recreational use with body contact.
Class III	Water Supply III – Extensive treatment required. Fishery III – Common, of economic value and tolerant species; livestock drinking.
Class IV	Irrigation
Class V	None of the above

Most stations in Sg. Pulai was categorized as Class III, which is common, has economic value, and is suitable for tolerant aquatic species culture (Table 3). This categorization was recorded in the wet (November to March) and dry seasons (May or early June to September (MET Malaysia, 2024)). Based on the readings obtained in the current study and earlier studies by the DOE, the water quality of Sg. Pulai was found to have deteriorated from 'Slightly Polluted' to 'Polluted' (Table 4).

Table 3: Classification of sampling stations according to specific parameters

Sampling stations	Water quality class according to specific parameter					
	Biological Oxygen Demand	Ammoniacal Nitrogen	Chemical Oxygen Demand	Dissolved Oxygen	pH	Total Suspended Solid
S1 (Sg. Senapang)	II	I	V	IV	I	III
S2 (Sg. Jelutong)	II	I	V	IV	I	III
S3 (Sg. Ulu Pulai)	III	I	V	IV	I	III
S4 (Sg. Jeram Choh)	III	I	V	IV	I	III
S5 (Sg. Jeram Batu)	III	I	V	IV	I	IV
S6 (Sg. Soka)	II	I	V	IV	I	III
S7 (Sg. Moleh)	III	I	V	III	I	IV
S8 (Sg. Redan)	I	I	V	III	I	III

Table 4: Comparision of Sungai Pulai's water quality classification from the Department of Environment (DOE) and FRI studies

Year	WQI	Class	Category	References
2016	68	III	Slightly Polluted	DOE
2017	64	III	Slightly Polluted	DOE
2019	72	III	Slightly Polluted	DOE
2020	80	II	Slightly Polluted	DOE
2022	52	III	Polluted	FRI
2023	54	III	Polluted	FRI

In conclusion, long-term monitoring of river water quality is essential to ensure clean water sources for human and animal use in the study area. In the future, the element of phytoplankton community structure as biological indicators for water quality will also be included. ■

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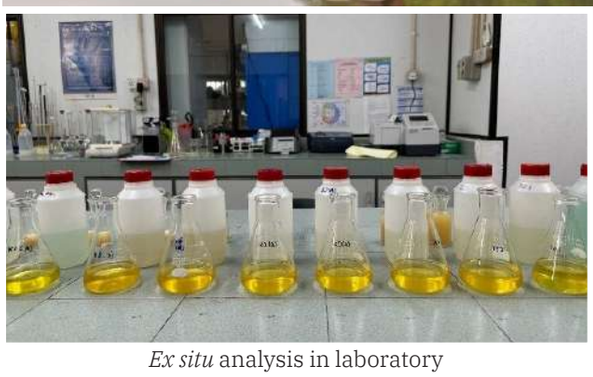
Sampling activity



Ex situ analysis in laboratory

Anisakis in Your Fish? Unseen Risk

ROHAIZA ASMINI Y[1]* & KUA BC[2]

[1] National Fish Health Research Center, 11960 Batu Maung, Penang
[2] Fisheries Research Institute, 11960 Batu Maung, Penang
rohaizaasmini@dof.gov.my

Anisakis is a roundwarm that lives in the digestive system of marine mammals and humans. *Anisakis* undergoes a complex life cycle that requires multiple hosts, such as fish and cephalopods as intermediate hosts and dolphins, whales, seals, and other marine mammals as final hosts (Fig. 1). *Anisakiasis* is a disease caused by accidental ingestion of third-stage *Anisakis* larvae (L3), which is linked with the consumption of raw, undercooked, marinated, pickled, smoked or salted fish or cephalopods. Two common species that cause human infection are *Anisakis simplex* and *Anisakis pegreffii* (Pozio, 2013). Thus, *Anisakis* larvae may pose risks to humans and impact the fishery product trade (Miguel et al., 2019). Several years ago, *Anisakis* spp. was detected in eight brands of canned fish products in Malaysia (ProMED, 2018), causing some commotion on social media.

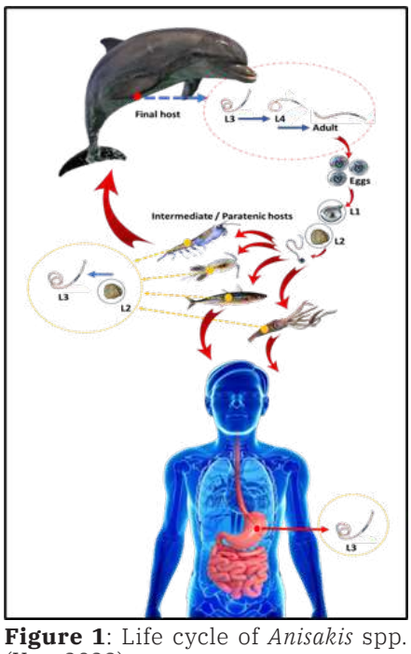


Figure 1: Life cycle of *Anisakis* spp. (Kua, 2023)

Common symptoms of anisakiasis in humans encompass nausea, bouts of vomiting, and discomfort in the upper abdomen, known as epigastric pain (Mattiucci, 2015). Infection can occur within hours of ingestion, as *Anisakis* larvae penetrate the mucosal layers of the gastrointestinal tract, causing tissue damage. Moreover, *Anisakis* is implicated in allergic reactions occurring after secondary exposure to the parasite, such as urticaria, angioedema, asthma, and, rarely, anaphylaxis in highly sensitized people (BIOHAZ, 2010).

Under the 12th Malaysia Plan, a project was carried out to determine the prevalence of L3 *Anisakis* sp. in *Decapterus* spp. (scad) along the west and east coasts of Peninsular Malaysia. The following three locations were selected: Kuala Terengganu, Terengganu; Bagan Panchor, Perak; and Kuala Perlis, Perlis. In this study, scad (n=30) were collected every month. The fish were dissected, and internal organs (gut, liver, pyloric cecum, intestine, and gonad) were removed and washed with saline water. The presence of L3 *Anisakis* sp. on each organ was observed and enumerated through the naked eye (Fig. 2). The individual larvae were collected and stored in a 1.5-mL tube containing 70% ethanol. The L3 morphology was examined under the microscope and later confirmed using a molecular method. The study shows the prevalence ranged between 3.33-86.7% at Kuala Terengganu, 3.33-80% at Bagan Panchor, Perak and 6.67-93.3% at Kuala Perlis.

Morphological and molecular analysis revealed that the L3 examined was *Anisakis typica*. Most L3 were observed in the peritoneal cavity, reproductive organs,

Infection can occur within hours of ingestion, as *Anisakis* larvae penetrate the mucosal layers of the gastrointestinal tract, causing tissue damage.

Figure 2: An image of *Anisakis* on internal organs of the scad

stomach, and pyloric caecum. No L3 were detected in fish muscle. These findings offer crucial insights into the prevalence of L3 *Anisakis typica* within *Decapterus* spp. In 2024, this project emphasises further ascertaining the presence of Anisakis nematodes in caged fish. This project is led by Dr. Kua Beng Chu and Ms Rohaiza Asmini (NaFisH), Ms Masazurah A. Rahim (FRI Batu Maung), Ms Noorul Azliana Jamaludin (FRI Kg Acheh) and Ms Annie Nunis Billy (MFRDMD) as co-researchers.

This study was supported by the Department of Fisheries through the Development Fund under Fish Health Research (P21300040170502) ■

Figure 3: Scad fish used for detection of L3 *Anisakis* sp

Under the 12th Malaysia Plan, a project was carried out to determine the prevalence of L3 *Anisakis* sp. in *Decapterus* spp. (scad) along the west and east coasts of Peninsular Malaysia.

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Unveiling the Mysteries: **Insights from Turtle Necropsies**

MOHD FATHULLAH R* & UMMI RODHIAH AB
Fisheries Research Institute Rantau Abang, 23000 Kuala Dungun, Terengganu
*fathullah@dof.gov.my

Turtles are crucial in maintaining ecological balance by controlling the jellyfish population, improving the ocean nutrient cycle and seagrass, and maintaining coral reef biodiversity (Teelucksingh et al., 2010; Lovich et al., 2018). Unfortunately, their existence is jeopardized by various human-induced pressures. Ironically, humans, who should act as nature's protector, contribute to the decline and demise of a species that predates us by thousands of years. Sea turtles should roam freely in the open ocean but struggle with habitat loss and degradation, climate change, pollution, by-catch, loss of nesting beaches, illegal harvesting, diseases, and other anthropogenic threats (Wilson et al., 2019).

The seriousness of these threats is reflected in the escalating number of deaths recorded in Malaysia. In Terengganu, deaths peaked at 71 in 2022 (Terengganu State Fisheries Office), an alarmingly high for a protected species under the International Union for Conservation of Nature (IUCN) red list.

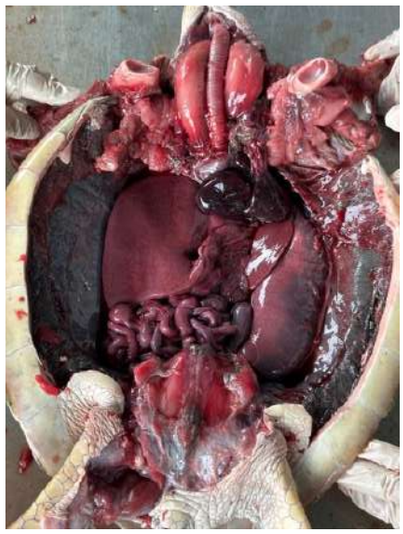
Usually, a stranded turtle was buried without proper investigation. The cause of death was determined merely through physical examination of the carcass without supporting biological or physiological data. A meticulous analysis of the causes of death is imperative to comprehend and address the issue of turtle stranding. Because of this issue, FRI Rantau Abang (FRIRA) has been improving the post-mortem examination procedures to include examination of tissues and organs to identify diseases, infections, or abnormalities that may led to their demise. Every single organ was taken out for observation; detailed conditions were recorded, and consultation with the veterinarian was conducted.

The inflamed part of the larynges

Sometimes, several signs, such as lesions, inflammation, and the presence of foreign matter that caused distress to the turtles could be easily observed.

From our experience, the main concern was the presence of foreign matters in the gastrointestinal tracts of the demised turtles. The turtles' omnivorous and non-selective feeding behaviour exposed them to ingesting diverse materials from the environment, including anthropogenic debris such as plastic. This finding agrees with Schuyler et al. (2014), who reported that nearly half of the post-hatchling loggerhead turtles studied contained ingested plastic.

A plausible cause is derived from necropsy for data analysis, yet a larger sample size is necessary to comprehensively understand and represent the actual threats faced by our national treasure. To this end, FRIRA implements necropsy networking strategies, collaborating with universities, agencies, and state fisheries to manage stranding cases. FRIRA has adopted necropsy networking strategies to achieve these strategies by collaborating with universities, agencies, and state fisheries to manage stranding cases. This collaboration unveils more stranding cases and facilitates more necropsies across Malaysia. A larger sample size is critical to generate scientific data addressing the severity of each threat to sea turtles in our national waters. This information aids state governments in devising effective mitigation strategies based on the threats identified through necropsy. Furthermore, additional exposure and training, particularly from veterinarians or marine biologists, are essential to empower our researchers with the knowledge needed to develop expertise in this field. ■



The inflamed part of the larynges

From right; fish bones, rubber fragments, plastic, ropes

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Exploring Coral Diversity and Abundance in Investigator Shoals, Spratly Islands

STEPHENIE D[1]*, MOHD F[2], DAUD A[1], MUSHIDI H[1] & MOHAMAD S[1]

[1] Fisheries Research Institute Bintawa, Jalan Perbadanan, 93744 Kuching, Sarawak

[2] Borneo Marine Research Institute, University of Malaysia Sabah, Port Bypass, 88400 Kota Kinabalu, Sabah

stephenie@dof.gov.my

According to the Reefs at Risk in Southeast Asia (RRSEA) project, nearly 4,006 km^2 of coral reefs can be found along the coasts of Peninsular Malaysia, Sabah, and Sarawak (Burke & Selig, 2002). Despite that, little is known about the coral reefs in the Spratly Islands. The Spratly Islands, named after Captain Richard Spratly, who sighted the island, are located in the southern part of the South China Sea and comprise reefs, shoals, atolls, and small islets. Most of the 'islands' are underwater during high tide, whereas some maintain a partial low elevation of a rim surrounding the lagoon. One of the shoals is the Investigator shoal or "Terumbu Peninjau'. This shoal is a large, submerged atoll with an area of approximately 205 km^2; it has a club shape and a narrow handle that points westwards (Hancox & Prescott, 1995). Malaysia first took possession of the feature in 1999 (Alexander, 2016). This shoal remains one of the dispute areas and is constantly claimed by China, Vietnam, and Philippines.

Located next to the Coral Triangle, this shoal is presumably rich in coral species diversity and considered one of the most crucial stocking, breeding, and nursing grounds for marine organisms (Konstantin et al., 2020). The reefs in Spratly Island were at risk due to destructive fishing practices, sedimentation, marine-based pollution, and overfishing based on RRSEA estimation (Burke et al., 2011). Nevertheless, geopolitical disputes have ignored attempts to declare the Spratly Islands as an international marine park (McManus et al., 2010).

The Fisheries Research Institute Bintawa, studied the coral diversity and abundance in Investigator Shoal. A total of 201 species of 69 genera and 24 families were recorded, with *Acropora* sp., *Tubipora* sp., *Porites* sp., *Pocillopora* sp., and *Goniastrea* dominating the area (Fig. 1, Table 1). Approximately 40 species were registered as the least common, according to Veron et al. (2016). According to the abundance status from the website of Corals of the World, the following five rare species were documented: *Astreopora cucullate*, *Pachyseris gemmae*, *Lobophyllia serrata, Oxypora echinata, Dipsastraea veroni* and *Platygyra yaeyamaensis* (Fig. 2). A total of 17 vulnerable (VU) and one endangered (EN) species were listed based on their conservation status from the IUCN Red List of Threatened Species.

In response to natural threats like bleaching events and associated disease outbreaks, the sustainability of coral reefs is at risk. Corals in the shoal showed some signs of compromised health and diseases (predation scar by fish grazing or COT, ulcerative white spot, pigmentation response, yellow blotch, and skeletal eroding band) (Fig. 3). This results in their morphological and physiological abnormalities caused by the biotic and abiotic stressors (Raymundo et al., 2008).

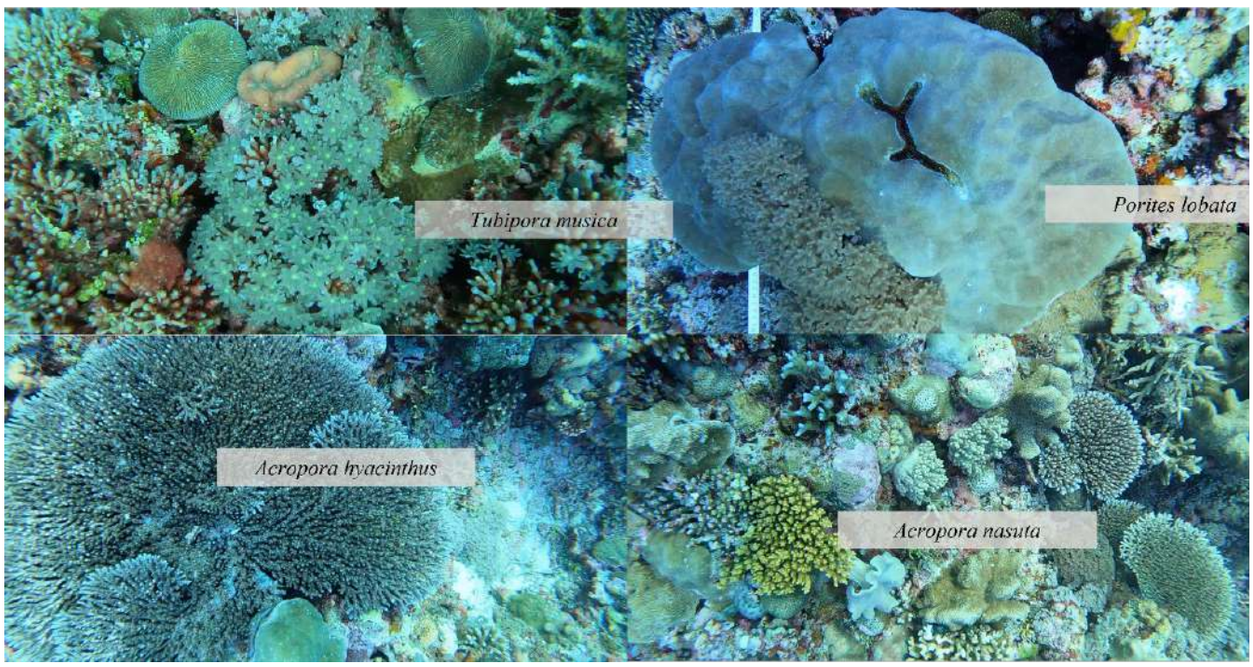

Figure 1: Dominant corals found in the Investigator Shoal

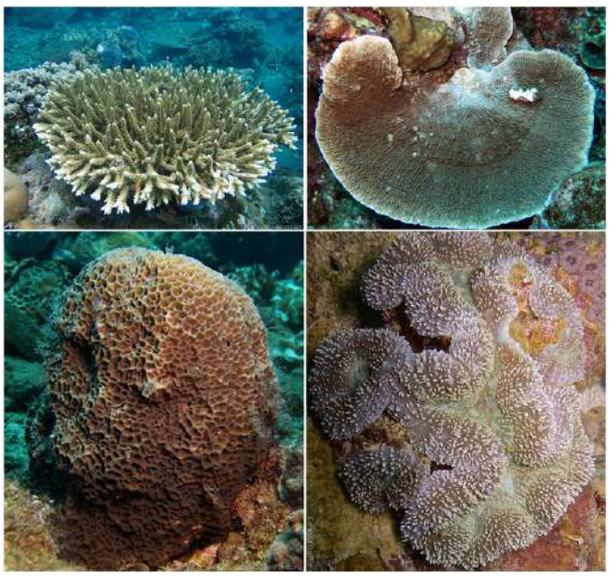

Figure 2: Some of the least common coral species discovered in the Investigator Shoal

In conclusion, this is the first record of coral status in Investigator Shoal. The corals are proven to possess massive biodiversity that is worth to be studied. This research provides updated data on coral diversity and abundance that the relevant agencies could use. Moreover, providing data to the Malaysian government will help strengthen Malaysia's claim over this disputed area. ■

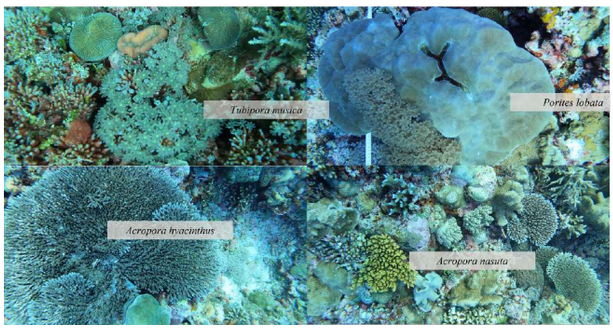

Figure 3: Coral diseases and signs of the compromised health of corals in the Investigator Shoal

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Table 1: Number of species, genera and families recorded at the Investigator Shoal

No	Family	Genus	S1	S2	S3	S4	S5	Mean (% ± SE)
	SCLERACTINIAN CORALS							
1	Acroporidae Verrill, 1902	*Acropora*	++++	++++	++++	++++	++++	**17.4 ± 0.5**
2		*Astreopora*	+	+	+	++	+	**0.9 ± 0.1**
3		*Isopora*	+	+	+++	++	++	**3.2 ± 1.7**
4		*Montipora*	++	++	++	++	++	**3.2 ± 0.4**
5	Agariciidae Gray, 1847	*Coeloseris*	-	+	+	+	-	**0.2 ± 0.1**
6		*Gardineroseris*	-	+	-	-	-	**0.0 ± 0.0**
7		*Leptoseris*	+	++	+	+	-	**0.4 ± 0.3**
8		*Pavona*	++	++	++	+	++	**1.9 ± 0.4**
9	Pachyseridae Benzoni & Hoeksema, 2023	*Pachyseris*	++	++	++	++	++	**1.8 ± 0.1**
10	Coscinaraeidae Benzoni, Arrigoni, Stefani & Stolarski, 2012	*Coscinaraea*	-	-	+	-	+	**0.1 ± 0.1**
11	Dendrophylliidae Gray, 1847	*Tubastrea*	-	+	-	-	-	**0.1 ± 0.1**
12		*Turbinaria*	+	+	+	-	-	**0.1 ± 0,1**
13	Diploastraeidae Chevalier & Beauvais, 1987	*Diploastrea*	+	+	+	-	++	**0.4 ± 0.2**
14	Euphylliidae Alloiteau, 1952	*Euphyllia*	+	-	-	-	-	**0.0 ± 0.0**
15		*Galaxea*	++	++	++	++	++	**1.4 ± 0.1**
16	Fungiidae Dana, 1846	*Ctenactis*	+	+	++	+	++	**0.8 ± 0.2**
56		*Porites*	+++	++++	+++	++++	++++	**11.7 ± 1,4**
57	Psammocoridae Chevalier & Beauvais, 1987	*Psammocora*	+	+	+	+	+	**0.2 ± 0.1**
	NON-SCLERACTINIAN CORALS							
58	Helioporidae Moseley, 1876	*Heliopora*	++	++	+	++	++	**1.1 ± 0.1**
59	Milleporidae Fleming, 1828	*Millepora*	+	+	+	-	+	**0.4 ± 0.2**
60	Tubiporidae Ehrenberg, 1828	*Tubipora*	+++++	++++	+++	++++	+++	**13.0 ± 2.9**
	SOFT CORALS							
61	Clavulariidae Hickson, 1894	*Clavularia*	-	+	-	-	-	**0.1 ± 0.1**
62	Nephtheidae Gray, 1862	*Dendronephthya*	+	+	+	+	-	**0.1 ± 0.0**
63		*Litophyton*	+	++	+	+	++	**0.6 ± 0.3**
64	Sarcophytidae McFadden, van Ofwegen & Quattrini, 2022	*Lobophytum*	++	++	++	+	+++	**1.2 ± 0.2**
65		*Sarcophyton*	++	++	++	+	+++	**1.5 ± 0.3**
66	Sinulariidae McFadden, van Ofwegen & Quattrini, 2022	*Sinularia*	+	++	++	+	+++	**1.2 ± 0.2**
67	Xeniidae Ehrenberg, 1828	*Xenia*	++	++	-	-	-	**0.8 ± 0.6**
68	Ellisellidae Gray, 1859	*Juncella*	+	-	-	-	-	**0.0 ± 0.0**

Note. +: One or few covering < 1% of coral colonies; ++: Uncommon covering 1 – 5% of coral colonies; +++: Common covering 6 – 10% of coral colonies; ++++: Abundant covering 11 – 20% of coral colonies; and +++++: Dominant covering > 20% of coral colonies.

Taxonomic Insight Into Sg. Kerian's Aquatic Ecosystem Using 18S Amplicon Sequencing

MASAZURAH AR* ROZIAWATI MR, MOHD NOR AZMAN A, NAJIHAH M, MUHAMMAD FAROUK H, INTAN NURLEMSHA B
Fisheries Research Institute, 11960, Batu Maung, Penang
masazurah@dof.gov.my

Sg. Kerian flows through the Kerian District in Perak, and the surrounding area is known for its agricultural activities, including aquaculture. The river and its surrounding landscape contribute to the economic and cultural aspects of the region. This study delves deep into the aquatic ecosystem of Sg. Kerian, to uncover detailed taxonomic information through 18S amplicon sequencing methodologies. The genetic information extracted through this method promises to reveal a wealth of knowledge about the diverse aquatic organisms. By harnessing cutting-edge molecular methods, this study aims to demonstrate the concealed narratives embedded in the genetic imprints of the myriad organisms, elucidating their contributions to the intricate fabric of Sg. Kerian's aquatic biodiversity

In molecular studies, different DNA markers serve distinct purposes. For instance, the 18S rRNA, for example, is commonly used for exploring the diversity of eukaryotic microorganisms, offering universality and conserved regions suitable for broad-scale studies (Kounosu et al., 2019). On the other hand, the 16S rRNA gene targets prokaryotes (Weinroth et al., 2022), the ITS region specific to fungi (Tedersoo et al., 2021), and the COI gene for animal identification (Petit-Marty et al., 2021). Plant studies often involve rbcL and matK genes (Nevill et al., 2020). Each marker has advantages and limitations, with choices depending on the organisms under investigation and the desired level of taxonomic resolution. Researchers may combine markers to achieve a more comprehensive understanding of biodiversity in various ecosystems.

The term "18S amplicon" refers to a specific DNA fragment that is amplified using the polymerase chain reaction (PCR) technique from the 18S ribosomal RNA (rRNA) gene. The 18S rRNA gene is a component of the small subunit of the ribosome and is found in the genomes of eukaryotic cells. It contains regions highly conserved across different species and variable regions that can be used to distinguish between taxa. The 18S amplicon sequencing has become a powerful tool in molecular ecology and biodiversity studies, allowing researchers to explore and identify various eukaryotic microorganisms in environmental samples. This technique enables a high-throughput analysis of complex microbial communities, offering insights into the diversity, abundance, and taxonomic composition of organisms such as protists, fungi, and metazoans. By sequencing the 18S amplicon, scientists can gain a comprehensive understanding of the microcosmic world within ecosystems, aiding in ecological assessments, biomonitoring, and the elucidation of community dynamics in various environmental niches (Trego et al., 2022)

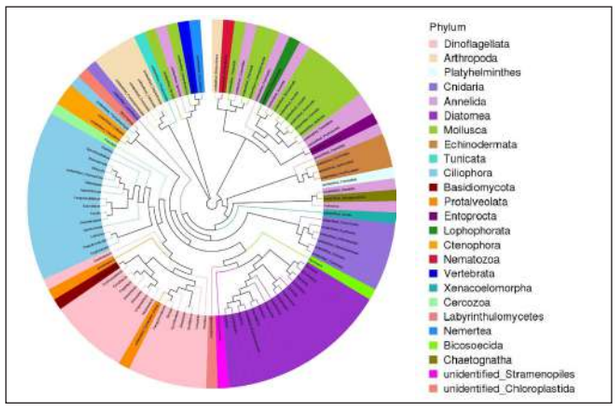

Figure 1: The evolutionary tree in the genus derived from water samples collected along Sg. Kerian. Different colors of the branches represent different phyla

As we delve into the depths of Sg. Kerian's waters, our focus encompasses the microscopic inhabitants, from Tunicata's pulsating life forms to Echinodermata's intricate beauty, the microscopic dance of Ciliophora, and the delicate intricacies of Cnidaria. We navigate through the sinuous forms of Annelida, the molluscan marvels, the flatworm mysteries of Platyhelminthes, the diatom delicacies of Diatomea, the dynamic dinoflagellates, and the resilient arthropods (Fig. 1). Join us on this scientific voyage as we decipher the genetic language that unveils the taxonomic intricacies of Sg. Kerian's aquatic inhabitants. ■

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Fisheries Research Institutes R&D Collaborations in the 12th Malaysia Plan

LIYANA R*
Fisheries Research Institute, 11960 Batu Maung, Penang
*liyana@dof.gov.my

Collaboration is critical in R&D as joining forces has been demonstrated to improve R&D output and outcomes. Researchers must maintain and reinforce interdisciplinary collaborations at the Fisheries Research Institute (FRI) to guarantee the long-term success of fisheries R&D. Thus, collaboration is fundamental at FRI, where researchers engage with a diverse array of stakeholders, including academics, private sector, individuals, farmers, and other interest groups for the prospects of knowledge sharing, expertise exchange, better comprehension of ecosystems, industrial evaluation and pre-commercialization. The top management recognizes the importance of collaboration between FRI and the Department of Fisheries. A policy and SOP on collaboration with outside agencies were recently endorsed to ensure smooth application processing and preparation of collaboration documents.

In the 12th Malaysia Plan, FRI received more than 50 collaboration applications, especially from universities and private companies (Figs. 1 and 2). Over half of the applicants were still trying to secure funding, whereas a few were still in the planning and discussion phases. Eight collaboration projects were finalized, endorsed, and materialized to sign the Memorandum of Understanding (MoU) or Memorandum of Agreement (MOA) (Table 1).

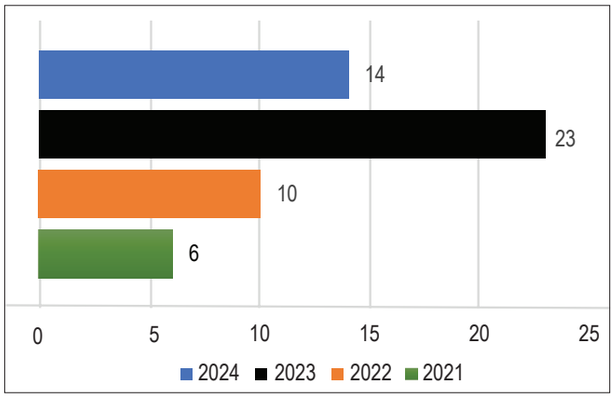


Figure 1: Number of Partner's Approaches (LOI)

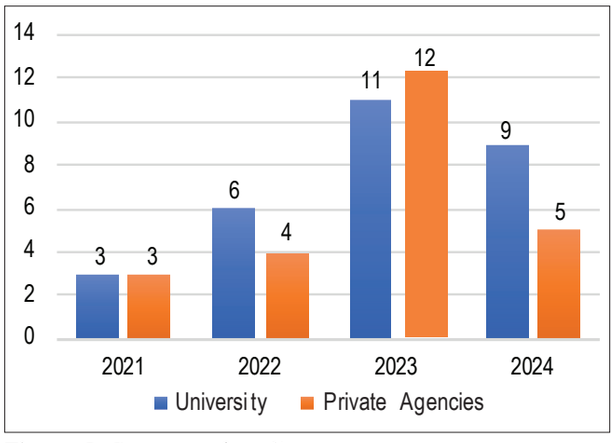


Figure 2: Category of applicants

Table 1: Number of MOU/MOA signed between FRI, DOF, and other establishments

Year	Collaboration document	Partner	Project Titles
2024	MOA	Biogenes Technologies Sdn. Bhd.	Memorandum of Agreement on Development of a Biosensor for Rapid Detection of Microsporidian Parasite Disease (*Enterocytozoon hepatopenaei*) in Marine Shrimp Farming
2023	MOU	Underwater World Langkawi	Technology Transfer, Information Sharing, and R&D Cooperation
2023	MOA	Dr. Mat Vet Sdn. Bhd.	Pre-commercialization of PrimEZeat Shrimp Breeding Wet Food
2023	MOA	Universiti Utara Malaysia	Protozoan White Spot Disease Detection in Marine Fish Using Artificial Intelligence Techniques.
2022	MOA	International Islamic University Malaysia	Fish Health Management Research and Development.
2022	MOU	International Islamic University Malaysia	The Development Study of the Early Warning System (EWS) of the Harmful Algal Bloom (HAB).
2021	MOU	International Islamic University Malaysia	The Study of Genetic and Molecular Approaches in Breeding, Nutrition and Diseases of *Pangasius nasutus*.
2021	MOA	Biogenes Technologies Sdn. Bhd.	The Study of Early Detection of Shrimp Diseases with Mobile Phone Applications.

To those interested in initiating R&D collaboration with FRI, please submit a letter of intent specifying the topic, the scope of collaboration, and the respective researcher you would like to collaborate with. The letter should be addressed to the Senior Director, Fisheries Research Institute. Our researchers and their fields of expertise are available on our website: https://fri.dof.gov.my/. ■

Bangkok strain, F1 generation

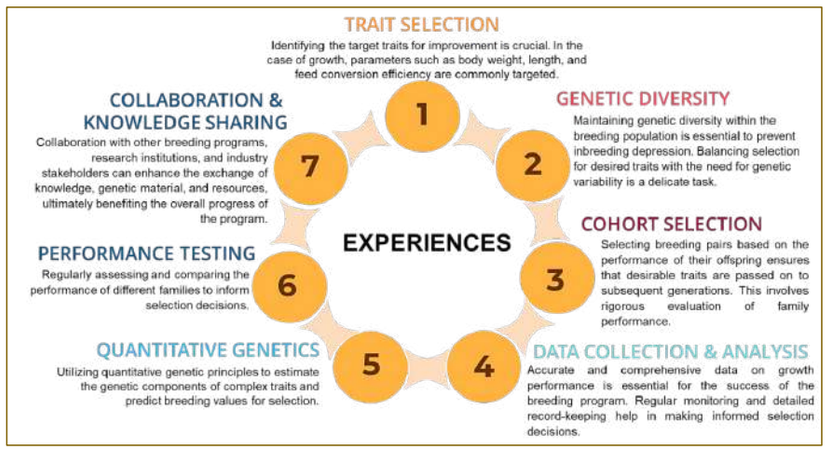
Selective Breeding Program of Asian Seabass (*Lates calcarifer*): Experience and Challenges

NUR FATIN AFIFAH OM* & SHAHARAH MI

Fisheries Research Institute Tanjung Demong, 22200 Besut, Terengganu

**fatinafifah@dof.gov.my*

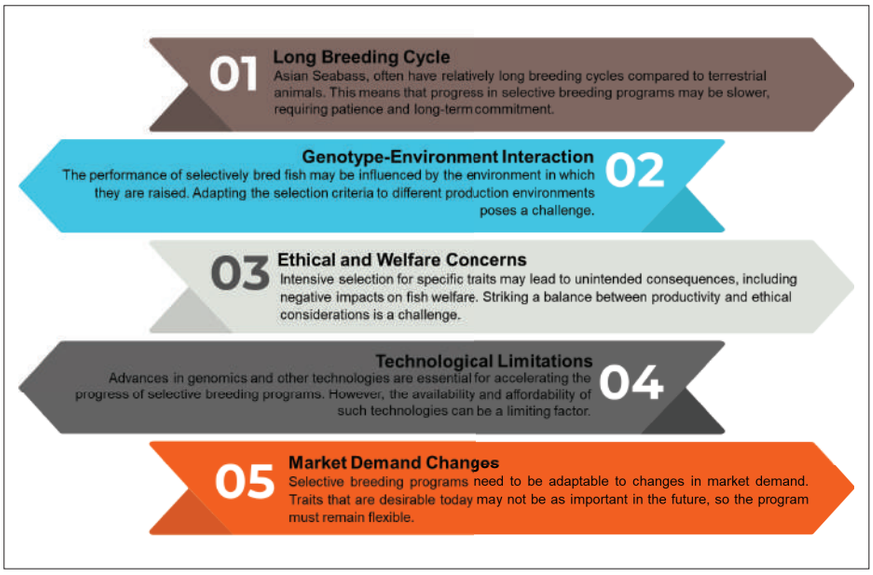
Selective breeding programs aim to improve specific traits in a population through controlled mating of individuals with desirable characteristics. In aquaculture species such as Asian seabass (*Lates calcarifer*), the goal is often to enhance traits such as growth rate, disease resistance, and overall productivity. The systematic breeding program of Asian seabass at the Fisheries Research Institute (FRI) Tanjung Demong, Terengganu, Malaysia, has entered its 9th year of implementation. Started in 11th MP, this program is continuously improvised in 12th MP under the Research, Development and Commercialization of Sustainable Aquaculture Technology Project, P21300040170501.



The selected broodstocks from Malaysia, Bali, and Bangkok were used as the base population to genetically enhance the production performance of Asian seabass. The programme has been produced until the F2 generation; the genetic information and growth performance of F1 and F2 are currently being collected and analyzed. The results obtained so far provide a tangible basis for improving the growth of Asian seabass in Malaysia. This improvement indicates ample opportunities for genetic improvement for the next generations of seabass, whereas genetic diversity among founder stocks could be exploited to enhance the growth traits.

Despite some initial achievements in this programme, the practical implementation of the breeding program for Asian seabass faced several challenges. Here are some key considerations, experiences, and demands associated with improving growth through a selective breeding programme conducted for Asian seabass.

CHALLENGES



Here are some key considerations, experiences, and demands associated with improving growth through a selective breeding programme conducted for Asian seabass.

PROVEMENT STRATEGIES:

- **Integration of Molecular Genetics**:

  Utilizing molecular genetic tools, such as DNA markers, can enhance the accuracy of trait selection and improve the efficiency of the breeding program.

- **Environmental Sensitivity Selection**:

  Considering the environmental conditions in the selection process helps develop fish that perform well across different farming systems.

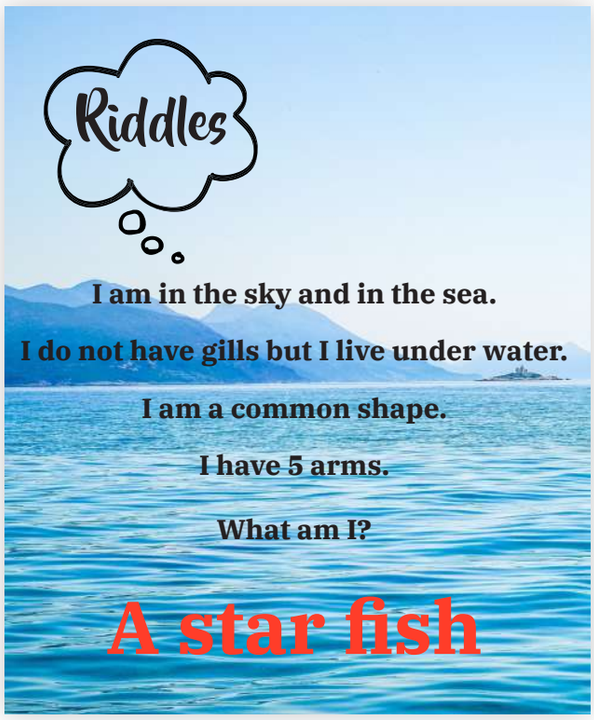
- **Incorporating Multiple Traits:**

  Besides growth, incorporating other economically important traits like disease resistance, fillet quality, and reproductive performance can lead to more well-rounded and resilient fish.

- **Selective Breeding Education:**

  Providing education and training to farmers and industry stakeholders about the benefits and principles of selective breeding can enhance the adoption and success of these programs.

In summary, a successful selective breeding program for Asian Seabass growth involves careful trait selection, genetic diversity management, data-driven decision-making, collaboration, and addressing challenges related to breeding cycles, genotype-environment interaction, and ethical considerations. The integration of advanced technologies and a focus on multiple traits contribute to the overall success and sustainability of the program. Ongoing research and collaboration within the aquaculture community are crucial for overcoming challenges and continuously improving breeding programs. ■



The Relationship Between **Seabirds and Fishing Gear**

RAJA HANIF ASYRAF RO*

Fisheries Research Institute Kampung Acheh, 32000 Sitiawan, Perak
**rajahanif@dof.gov.my*

Do you know that over 300 species of seabirds can be found worldwide? They comprise the smallest storm petrels (only a few grams) to the largest albatrosses (wingspan over 3 meters). The taxonomic orders of seabirds are Procellariiformes (albatrosses, petrels, shearwaters, and fulmars), Pelecaniformes (pelicans, cormorants, and gannets), Charadriiformes (gulls, terns and auks) and Sphenisciformes (penguins).

Seabirds are adapted to live in the harsh marine environment by having unique characteristics, including the ability to excrete excess salt through supra-orbital salt glands, pursue prey through diving, possess excellent amphibious vision enabled them to make extensive use of the marine environment for at least part of their annual cycle (Croxall et al., 2012). However, most seabirds exhibit high adult survival, slow maturation, and low annual reproductive output, indicating a significant investment in parental care that does not come at the cost of future survival and making birds reliable biomarkers for the health of fish populations (Velarde et al., 1994).

Seabirds are often attracted to fishing vessels due to the abundance of food, such as fish discards, and the opportunity to scavenge for food. This attraction is fatal as it increases the risk of by-catch and being killed by fishing gear. By-catch refers to the accidental capture of non-target species in fishing gear. According to Dias et al. (2019), nearly 30% of all seabird species are affected by by-catch, resulting in hundreds of thousands of deaths yearly (Žydelis et al., 2013). Using fishing gear designed for specific fish species has been linked to the high mortality rates of seabirds globally. According to Kuemmerlen et al. (2016), gillnet fisheries in the North Sea have been found to have a significant by-catch rate of seabirds, with over 10,000 birds being caught annually, and Abraham & Thompson (2011) mentioned that most of these birds were seen during longlining activities.

Seabird by-catch in fishing operations is a major threat to vulnerable bird populations. Thus, there is an urgent need for effective by-catch reduction strategies to protect these species. This issue has been addressed by implementing regulations and using mitigation measures. For instance, bird-scaring lines, weighted lines, night settings and hookpod effectively reduce seabird by-catch. These mitigation measures have successfully limited the number of seabirds caught in fishing operations, highlighting their importance in preserving these vulnerable bird populations.

The interaction between seabirds and fishing gear is a complex issue that requires a delicate balance between conservation efforts and the needs of fishermen. However, it is also essential to consider the economic and social impacts of implementing strict regulations on the fishing industry, as fishing gears provide food security for millions of people worldwide. Therefore, it is crucial for continued research and collaboration between conservationists and fishermen to find sustainable solutions that protect seabird populations while also supporting the fishing industry. This requirement requires a comprehensive understanding of the impact of fishing gear on seabirds and finding new alternative fishing methods and mitigation measures that minimize harm to the seabirds. ■

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The First Record of the Pteropod, *Creseis acicula*, from the Coast of Mersing, Johor

MS ISMAIL[1]* & NURHUDA I[2]

[1] National Marine Park Research Centre, Fisheries Research Institute, 11960, Batu Maung, Penang
[2] Johor State Fisheries Office, Gelang Patah 81550, Johor
saupi@dof.gov.my

Marine pelagic gastropods comprise a relatively poorly known group in the Malaysian waters, especially along the coast of Johor, with only a few species recorded to date. *Creseis acicula* (Rang, 1828) is a warm-water pelagic macro-zooplankton widespread worldwide. Its blooms have been reported in China, India, Japan, Mexico, Turkiye, and the Mediterranean seas. However, until 2024, this sea butterfly was never recorded in the Malaysian waters. Here, we report the first outbreak of *C. acicula* off the coast of Mersing, Johor. Although there were previous occurrences of this straight-needle pteropod in Malaysia, this is the first reported pteropod bloom in the waters of Johor.

Live specimens were collected near the sea surface during two field trips to Pulau Mertang (11 and 12 June 2024) and one trip to Penyabong Beach (25 July 2024). The specimens were photographed and brought to the state Biosecurity Laboratory for further analysis. Underwater surveys around Pulau Mertang revealed many dead *C. acicula* buried inside barrel sponges. Species identification was performed according to Zhanhui et al. (2021) and SeaLifeBase (www.sealifebase.se). This species has a delicate, transparent, tube-shaped needle-like shell, with two distinctive 'wings' that project from the shell aperture. The collected samples have an average total length of 1.5–2 cm.

Identification under the microscope

Creseis acicula size compared to a 5-cent coin

Pteropod specimen collected at Mersing

The first bloom persisted for nearly a month, causing a minor setback to the tourism activities around the Mersing archipelago; hence, it has attracted serious attention from the local authorities and the public. We believe that increased water temperature, pH level, salinity fluctuation, and food availability are factors triggering this event. Thus, further studies should be carried out with the possibility of a future surge of *C. acicula*. In addition, this species could serve as one of the biological indicators of environmental or climate change. ■

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Wild and Cultured Sea Grape (*Caulerpa lentillifera*): What Are the Differences?

NIK NAZLI EFFENDY R
Fisheries Research Institute Pulau Sayak, 08000 Kota Kuala Muda, Kedah
**niknazli@dof.gov.my*

Sea grape (*Caulerpa lentillifera*) is one of the edible seaweed belonging to the *Caulerpaceae* family. They are often called green caviar because of their appearance, like fish roe. The glistening nodules are the leaf of the *C. lentillifera*. This seaweed is highly sought after for its soft and succulent texture and is traditionally eaten in Southeast Asia, Oceania, and East Asia. Sea grape is usually eaten raw and tastes like the ocean, with a soft texture that pops nicely in the mouth. Traditionally, they were harvested from the wild, but now, they are commercially farmed in ponds. Not only tasty, but sea grape also contains proteins, minerals, dietary fibre, vitamins, and saturated and unsaturated fatty acids.


Wild sea grape


Cultured sea grape

C. lentillifera grows well in various environments, from coral rubble or rocks over 50 meters deep to shallow and muddy lagoons. It is commonly found on sandy to mussy substrates on reef flats that are not exposed during low tides and where the water is calm. *C. lentillifera* is stenohaline and cannot thrive in areas with less than 25% salinity.

In the 12th Malaysia Plan, R&D on *C. lentillifera* culture in various systems was initiated at the FRI Pulau Sayak, Kedah, to produce the best yield and quality. This article highlights the difference between wild and cultured sea grape used in this project. Wild sea grape denotes the one that grows in open water, in this case from Port Dickson, Negeri Sembilan, whereas cultured sea grapes pertain to those cultured under a controlled environment at FRI Pulau Sayak.

These two groups have few significant differences, particularly in the availability, quality, and nutritional value.

- **Availability**

Wild sea grapes are available from May to August yearly, whereas cultured ones are available all year round. Global warming may affect wild *C. lentillifera* and contribute to its distribution and existence. Besides that, wild sea grapes are affected by salinity, which explains why there is no wild seaweed during the rainy season (Zuleyma et al. 2016).

- **Quality**

The quality of *Caulerpa* depends on the habitat in which they were found. Normally, wild sea grapes are dirty, covered with mud, and may harbour small creatures such as baby crabs, snails, worms, sea urchins, and sand aiptasia. The colour is uneven, with some parts appearing darker than others. As a bio-filter, the sea grape will absorb various substances in the water, including bacteria and heavy metals. Several studies have recorded higher concentrations of heavy metals and bacterial counts in wild *Caulerpa* compared to cultured ones. The cultured sea grape's colour is lighter green, monochromic, shiny, and clean.

• Nutritional Value

Table 1 presents the chemical composition of wild and cultured sea grapes. The wild sea grapes had higher protein, fibre, and moisture content than the cultured ones. According to Alfonsina (2018), the protein level generally depends on the species, season, and geographical distribution. Both wild and cultured sea grapes are high in calcium.

Table 1: Proximate and mineral composition of fresh *C. lentillifera*

Proximate analysis	Cultured	Wild
Crude protein (% w/w)	0.6	0.9
Crude fat (% w/w)	0.0	0.0
Crude fiber (% w/w)	0.7	0.9
Moisture (% w/w)	94.8	94.5
Ash (% w/w)	2.8	2.7
Calcium (mg/kg)	458.0	432.3
Phosphorus (% w/w)	0.014	0.006

#Method: AOAC 988.05 17th Edition

Wild sea grapes are more challenging to harvest. They were usually buried in the mud and needed to wait for low-tide water to be collected. For deeper water, the operators need to dive underwater to collect them. On the other hand, harvesting cultured sea grapes is straightforward and hassle-free.

In conclusion, wild or cultured sea grapes are economically essential commodities and should be strategically managed to ensure availability and sustainability. To ensure that the sea grapes farmed meet safety standards, they must be cultivated in areas accessible from pollution or less polluted areas. ■

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Wild sea grapes harvesting

Cultured sea grapes harvesting

Initial *Stichopus horrens* Biomass Calculation Using Leslie's Population Estimation Method at Pulau Pangkor, Perak

ZAIDNUDDIN I*, MD NIZAM I & MS ISMAIL
Fisheries Research Institute, 11960, Batu Maung, Penang
**zaiali01@dof.gov.my*

Populations of *Stichopus horrens* often face overfishing challenges, and efforts to mitigate this pressure require prolonged timelines or are deemed unfeasible. This study aimed to assess the population status of *S. horrens* in Pulau Pangkor, Perak, and proposed suitable conservation measures. The habitat of *S. horrens* in the vicinity of Pulau Pangkor encompasses Pulau Pangkor, Giam, Mentagor, and Pangkor Laut (Fig. 1). Fishing activities in Pulau Pangkor waters are conducted monthly by coastal fishermen through the free diving method. Collection occurs during low spring tides, typically 1–3 hours before high tide, and is limited to 5–7 days each month, depending on tidal and weather conditions at sea. This collection method was approximately 21 hours per month (3 hours × 7 days). Notably, the local community unanimously prohibits using scuba or hookah equipment for collection.

This study employed the Leslie regression or elimination model (Leslie & Davis, 1939). The underlying assumption was that the decrease in resource numbers resulted from fishing activities (Miller & Mohn, 1993; Akamine et al., 1992; Hart & Gorfine, 1997). The study aimed to be a trailblazer in investigating *S. horrens* populations to prevent overexploitation. The Leslie regression model was used to estimate the minimum catch population size of *S. horrens* in the waters of Pulau Pangkor. This model establishes a relationship between catch per unit effort and stock abundance (Knight & Cooper, 2008) under certain assumptions: (1) a closed population; (2) constant probability of each individual being caught in the trap throughout the experiment; and (3) all individuals having the same probability of being caught in sample t. Under these assumptions, a regression plot of catch-per-unit-effort (Ct/ft, Y-axis) against cumulative catch (Kt, X-axis) results in a straight line. In the Leslie regression model equation, the slope of the regression line represents the estimate of catchability, q. The Y-axis intercept is the product of the original population and catchability, q. The confidence limit for the q estimate equals the slope (b).

The data collected revealed a declining trend in the catch following successive collections. On the initial day of June at Pulau Pangkor Laut, the catch was 9.8 kg, 3.3 kg on the second day, and 0.9 kg on the third day. In July, the first day's collection at Pulau Mentagor amounted to 21.2 kg, with subsequent days yielding 7.6 kg and 1.9 kg, respectively. Moving to August at Batu Yasin, the first collection weighed 11.9 kg, followed by 7.8 kg on the second and third days, maintaining a catch of 3.1 kg. September's data at Kampung Teluk Gadong recorded 14.4 kg on the first day, 4.9 kg on the second day, and a minimal 0.3 kg on the third day. The cumulative catch for all these instances is illustrated in Fig. 2. The data indicated a swift decline in *S. horrens* collection when conducted regularly. Within the three-day period, the *S. horrens* stock diminished by 96% compared to the total catch on the first day. Given the slow movement of *S. horrens* from one area to another, the assumption was made that there was no new recruitment in the study area during the study. Pooling data from the four study areas yielded an initial population biomass of 92.03 kg (Table 1 and Fig. 3). Dividing the total weight of 92,031 g by the average weight of one *S. horrens*, which was 88.7 g (Zaidnuddin's unpublished data), the calculation yielded 1,038 individuals in a 400 m^2 area, equivalent to 2.59 individuals per square meter (ind/m^2).

Table 1: Cumulative catch for every first, second, and third sampling

t	Catch kg	boat	diver	C/f	SE	Lt (X)
1	57.311	4	8	7.16	2.47	00
2	23.67	4	8	2.96	1.10	57.31
3	6.24	4	8	0.78	0.61	80.98

Given the slow movement of *S. horrens* from one area to another, the assumption was made that there was no new recruitment in the study area during the study.

S. horrens collected by fishermen

Figure 1: Location of the capture attempt using the sampling method without returning the sample (Non-return sampling) (Google, 2024)

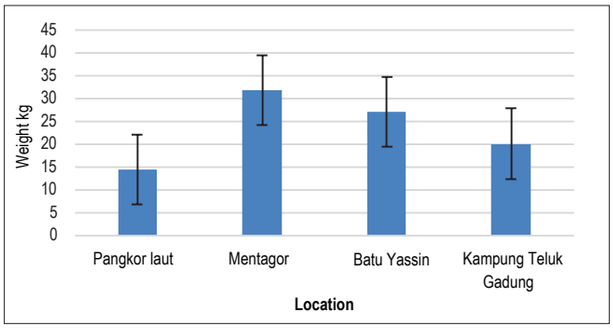

Figure 2: Total Sea cucumber collected at each location

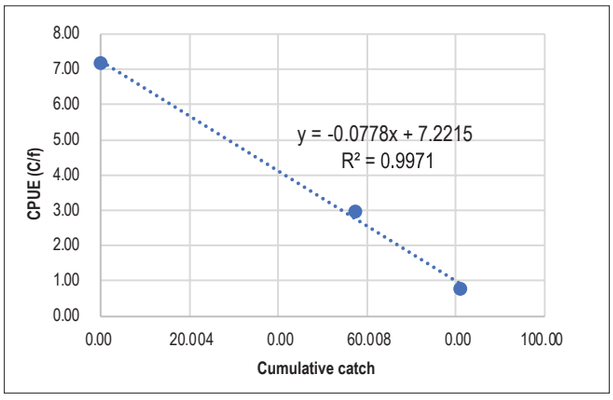

Figure 3: Linear Regression Graph of cumulative catch (Kt) and CPUE

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A Summary of Fish Landings
and Species Composition using Fish Aggregating Devices at Miri Waters, Sarawak

QHAIRIL SHYAMRI R*, MOHAMMAD HAFIZ H, NURRIDAN AH & JAMIL M

Fisheries Research Institute Bintawa, Jalan Perbadanan, 93744 Kuching, Sarawak
qhairils@dof.gov.my

Yellowfin Tuna captured using FAD

FAD deployed at Miri waters, Sarawak

Tuna fishery is a significant fishery resource in Malaysia regarding landings and values. There are two major groups of tuna, i.e., oceanic and neritic. The most exploited species among the oceanic tuna include Yellowfin (*Thunnus albacares*), Skipjack (*Katsuwonis pelamis*), and Kawa kawa (*Euthynnus affinis*) are the most exploited species whereas neritic tunas are the Frigate Tuna (*Auxis rochei*) and Bullet Tuna *(Auxis thazard*).

In Sarawak, tuna exploitation is limited to shallow inshore waters due to the lack of stakeholders joining this sector. Another main reason is due to the lack of Fish Aggregating Device (FAD), or '*payao,*' being deployed in Sarawak waters to aggregate these tunas and the lack of expertise by the local fishermen to operate purse-seine harvesting of FADs in Sarawak. FAD is a man-made structure that suspends a coconut frond in the water column to create new habitats for the smaller prey and fry. An abundance of food attracts the tuna species to feed in the vicinity of FAD, as tunas are considered a predatory species. The deployment of FAD attracts and aggregates pelagic fish in a certain area (Barut, 2003).

Eight FADs were deployed between June 2022 and June 2023 in Miri waters at depths ranging from 700 to 1200 meters. The FAD designs were developed by the Engineering Division, Department of Fisheries Malaysia. Sample collection was conducted using handline and traditional fishing methods, carried out by seven small boats operated by a fisherman. Handline or traditional fishing was conducted near FADs, targeting large-sized and deep-swimming tuna species such as the Yellowfin Tuna. Fishing operations were conducted within a 500-meter radius of each respective FAD. Upon capture, species identification was done following the method of Kheok & Gambang (2009). External morphological measurement was taken for Total Length (TL) using a set ruler.

Seven fish species were captured throughout the study, and total landings accumulated to 9.72 metric tonnes (MT). The most dominant tuna species was represented by Yellowfin (4.497 MT), followed by Skipjack (3.368 MT). A total of 1,667 Yellowfin tuna individuals were analysed using FISAT software and showed the value of $L\infty$ = 1904.7, K = 0.44, Z = 1.43, M = 0.31, F = 1.12, E_{crit}0.78, E_{10} = 0.335, E_{50} = 0.302 and E_{max} = 0.456. Other significant results include a significant difference between the density of Yellowfin Tuna captured in both 'payao' deployed at depths less than 1000 meters and more than 1000 meters with p-value = <0.001 based on independent *t*-test analysis conducted.

In conclusion, Yellowfin tuna resources in Miri waters are currently underexploited. Further deployment of FADs should be conducted to increase the oceanic tuna capacity in Sarawak waters. This deployment will help improve Malaysia's total landings, ensuring the nation's food security. ■

Table 1: Species checklist and landings for respective species in Miri waters, Sarawak, for 2022 and 2023

No.	Species	Landings in metric tonnes (MT)							Total (MT)
		July 2022	Sept 2022	Oct 2022	Jun 2023	July 2023	Sept 2023	Oct 2023	
1.	Yellowfin Tuna (*Thunnus albacares*)	0.01	1.11	1.15	0.42	0.34	0.99	0.48	4.49
2.	Skipjack Tuna (*Katsuwonis pelamis*)	0.64	0.35	0.16	0.31	0.22	1.56	0.12	3.36
3.	Kawa-kawa (*Euthynnus affinis*)	0.20	0.25	-	-	-	-	0.51	0.96
4.	Black Marlin (*Makaira indica*)	-	0.18	0.36	-	-	0.20	0.03	0.77
5.	Mahi-mahi (*Coryphaena hippurus*)	-	0.01	0.01	0.03	-	0.01	0.03	0.09
6.	Wahoo (*Acanthocybium solandri*)	-	-	-	0.01	-	-	0.03	0.04
7.	Blacksail Snake Mackerel (*Thyrsitoides marleyi*)	-	0.01	-	-	-	-	-	0.01
	TOTAL	**0.85**	**1.91**	**1.68**	**0.77**	**0.56**	**2.76**	**1.20**	**9.73**

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The Effect of Hydrolyzed Feather Meal-Based Diets on Growth Performance of Pacific Whiteleg Shrimp (*Litopenaeus vannamei*)

MOHD FIRDAUS A* & MOHAMMED SUHAIMEE AM
Fisheries Research Institute Pulau Sayak, Kedah
*firdausazmi@dof.gov.my

This study was conducted under the 12th Malaysian Plan- Research, Development, and Commercialization of Sustainable Aquaculture Technology (P21300040170501). The study aimed to assess the growth performance of whiteleg shrimp (*Litopenaeus vannamei*) fed with hydrolyzed feather meal (HFM) in earthen ponds during a 90-day feeding trial. The HFM is high in protein, particularly rich in cysteine, but deficient in lysine and methionine contents. HFM has emerged as an essential ingredient for ruminant animals and has also been demonstrated to be a good candidate to replace fish meal in fish feed (Cheng et al., 2002; Campos et al., 2017).

The HFM was procured from a local supplier in Johor, along with other ingredients. The FRI Pulau Sayak formulated the experimental diet and produced it in bulk (approximately 2,000 kg) by a private feed miller. The HFM replaced approximately 33.6% of the fish meal in the formulation. The experimental diet was kept isonitrogenous and isolipid at 40% protein and 8% lipid to the control diet (commercial pellet). A private company in Banting, Selangor implemented the feeding trial. Two earthen ponds (0.5 hectares) were used and prepared according to standard practices. Commercial probiotics and waste sludge reducer were applied as a biofloc starter. Ju et al. (2009) suggested that microalgae in the microbial floc may be crucial in improving shrimp growth rates. Shrimp juveniles (0.0035 ± 0.0003 g) were stocked at a density of 50/m^3, fed four times a day, and weighed once a month.

The growth performance of shrimps is shown in Fig. 1. There was no significant difference in the initial body weight ($P > 0.05$) of shrimps fed with an experimental or commercial diet at the beginning of the experiment. There were also no significant differences ($P > 0.005$) in final body weight or weight gain after 90 days of culture between the shrimps given the experimental diet and commercial diet. (Table 1). However, the specific growth rate for the shrimps fed with the commercial diet was slightly higher (9.75) than the experimental diet (9.71). In this trial, the feed conversion ratio (FCR) for the commercial diet was lower (1.30) compared to the experimental diet (1.50). This result suggested that replacing 33.6% fish meal with HFM in the white leg shrimp diet had no negative impact on growth performance and nutrient utilization. ■

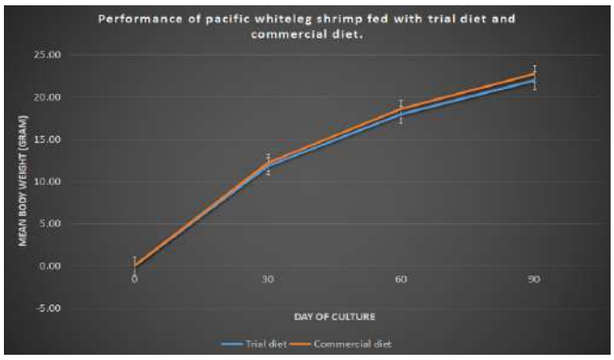

Figure 1: The growth performance of shrimp fed with 33.6% hydrolyzed feather meal and a commercial diet for 90 days of culture

Table 1: Performance of pacific whiteleg shrimp with commercial and experimental diet after 90 days of feeding trials.

	The diet used in the experiment	
	Commercial diet	Hydrolyzed feather meal (33.6%)
Initial mean weight (g)	0.0035±0.0003[a]	0.0035±0.0003[a]
Final mean weight (g)	22.71±2.23[a]	21.92±2.96[a]
Weight gain (%)	99.98±0.0017[a]	99.98±0.0028[a]
Specific growth rate (% day^{-1})	9.75	9.71
Feed conversion ratio (FCR)	1.3	1.5
Survival rate (%)	58.39	53.40

*The same superscripts indicate no significant differences ($p>0.05$) between the values.

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The Effect of Cinnamon Oil Incorporated Diet on the **Specific Growth Rate of Giant Freshwater Prawn (*Macrobrachium rosenbergii* (De Man, 1879))**

IFTIKHAR AAR[1]*, KUA BC[2], MAZLINI O[1] & NUR FATIHAH H[1]

[1] Fisheries Research Institute Glami Lemi, Jelebu, 71650, Negeri Sembilan
[2] Fisheries Research Institute, 11960, Batu Maung, Penang

**iftikhar@dof.gov.my*

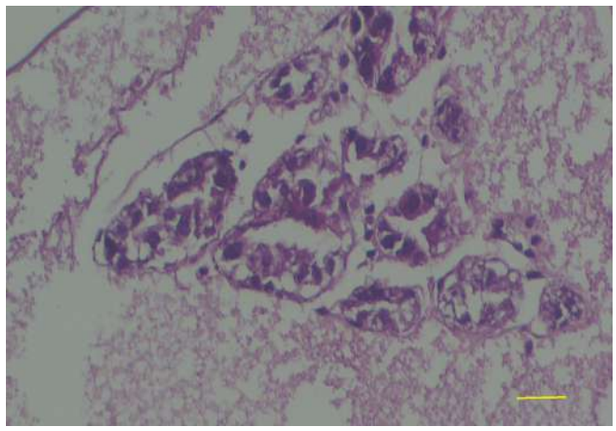
The giant freshwater prawn (GFP), *Macrobrachium rosenbergii*, is commonly farmed throughout Asia and the Pacific. In Malaysia, 183.15 metric tonnes of GFP were produced from aquaculture in 2022 (DOF, 2022), a slight decrease from 207.02 metric tonnes in 2021. One of the main constraints was the lack of good quality seed stock and high mortality during the juvenile stage (Mohamed et al., 2017). Essential oil (EO) as a supplement in the GFP diet enhanced growth and non-specific immunity in GFP (Bandeira et al., 2022; Liu et al., 2022).

In the 12th Malaysia Plan (Project: P300040170502: Research and Development in Aquaculture Fish Health), a study was carried out to investigate the effects of cinnamon EO (CIN) incorporated diet on the GFP-specific growth rate (SGR). The GFP post larvae (PL) were stocked (3 individuals/L) in 2-tonne rectangular tanks. Two experimental trials were executed in which PL received feed mixed with CIN and a controlled diet (without CIN) and were fed twice daily. The specific growth rate (SGR) was determined by Paul et al. (2016). GFP was also screened for *M. rosenbergii* nodavirus (MrNV), Infectious Hypodermal Haematopoiteic Necrosis Virus (IHHNV), and the histology samples was taken on day 0 and day 22 post-experiment. Water quality parameters were analyzed using the Hach kit and DR1900 spectrophotometer.

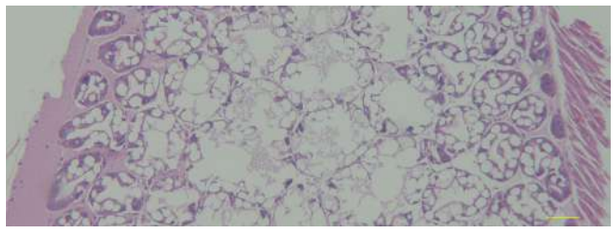
The results showed a notable increase in SGR in GFP fed with CIN (8.41%) compared to control (5.00%). The final GFP average weight in the treatment tank was significantly higher (0.19 ± 0.09 g) compared to the control (0. 009 ± 0.04 g) despite having the same initial weight (0.03 g). Histology analysis (Figs. 1 and 2) also showed improved lipid content in hepatopancreas organs in GFP receiving CIN diet. The study found that the prawn survival rate was lower in the group receiving CIN (72.66 ± 5.93%) compared to the control (86.25 ± 8.27%) (Table 1). Analysis of viral diseases, specifically targeting MrNV and IHHNV, yielded negative results for both groups of prawns. These results suggest that the use of cinnamon essential oil may have a positive impact on fish growth performance. ■

Table 1: Survival rate and weight of *M. rosenbergii* during the experiment

	Day of Culture 1		Day of Culture 22		Specific Growth Rate (SGR)
	Survival rate (%)	Average weight (g)	Survival rate (%)	Average weight (g)	
Control	100	0.03 + 0.01	86.25 + 8.27	0.09 + 0.04	5.00%
CIN	100	0.03 + 0.01	72.66 + 5.93	0.19 + 0.09	8.41%



Histology of prawn in control tanks (scale bar = 250 µm)



Histology of prawn in treatment tanks (scale bar = 250 µm)

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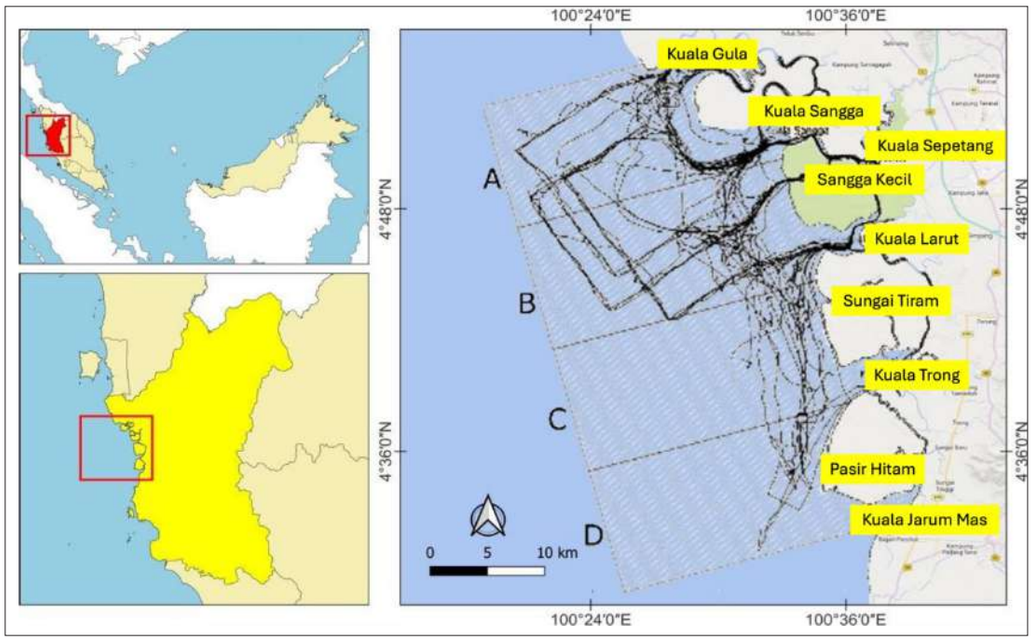

Figure 1: The survey areas in Matang waters, Perak, which were categorized into sites A (Kuala Sangga), B (Kuala Larut), C (Sg. Tiram river mouth till Kuala Trong), and D (Pasir Hitam & Kuala Jarum Mas' water)

Mapping the Prime Habitat of Indo-Pacific Humpback and Irrawaddy Dolphins in Perak Waters

MUHAMMAD AMIRUL SIDDIQ AR*

Fisheries Research Institute Rantau Abang,
23000 Kuala Dungun, Terengganu
*amirulsiddiq@dof.gov.my

The waters around Malaysia host 27 marine mammal species, with 23 confirmed to inhabit or traverse Malaysian territory and Exclusive Economic Zone (EEZ) waters (Jaaman, 2010). For instance, the Matang coastal waters in the state of Perak are home to both the Indo-Pacific humpback dolphin *(Sousa chinensis)* and the Irrawaddy dolphin *(Orcaella brevirostris)* (Kuit et al., 2014), which are known to adapt to various coastal environments and found across warm-temperate waters. The Matang mangroves and coastal waters are an extensive area comprising mangrove forests within a series of riverine and estuarine waterways that lead out into a shallow but productive intertidal mudflat coastal region. The mangrove forest was notable for having direct and indirect effects on the livelihood of megafauna (Sievers et al., 2019). Matang waters reside in the Matang Mangrove Forest Reserve, which has a high number of nutrient discharges that support primary production for the fish that eventually serve as prey for the dolphins (Chew et al., 2012), thus making it a hotspot and vital foraging area for the dolphins. A survey was carried out in 2022 to record the sighting of dolphins and their behavior. A precision area of 1 km × 1 km was employed in this survey (Becker et al., 2018).

The sighting sites of the two dolphin species were varied. The Indo-Pacific humpback was more commonly sighted further north in the Kuala Sangga area (A), whereas the Irrawaddy dolphin was frequently observed towards the south, Kuala Larut (B).

Although site A was crowded with moving ships, this did not bother the Indo-Pacific humpback dolphin. They ignored the ship and foraged the area exclusively. This dolphin species was also found deep inside the Sg. Matang area near Kuala Sepetang, which is full of moving vessels and human activities. The humpback dolphins were also friendly with the survey boat and took no account when the survey boat moved closer. By contrast, Irrawaddy dolphins were jittery, eluded the survey boat, maintained around 100 meters from the boat, and dashed away when they felt threatened. The Irrawaddy dolphin tended to forage in more pristine areas with less human interaction, such as in B, C, and D, as described by Pazi et al. (2021). The survey discovered that both species have different preferences for habitat and reactions toward human activities, as previously reported (Jiang et al. 2019).

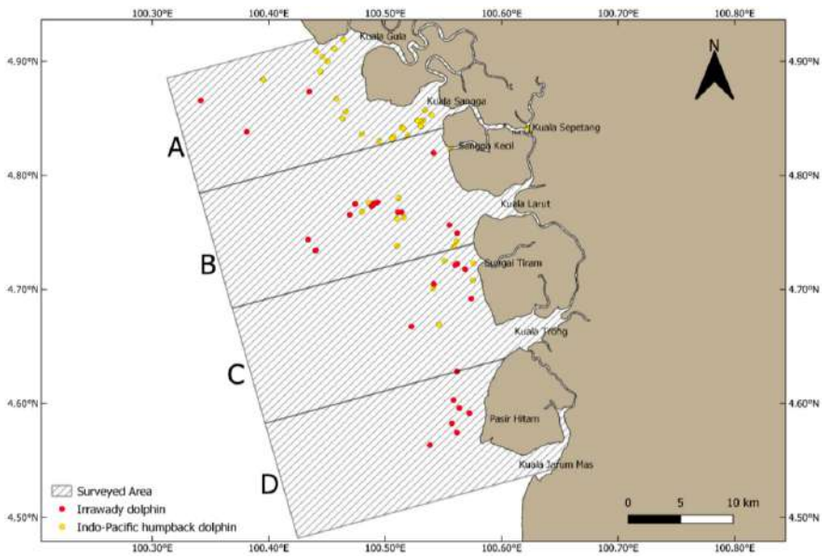

Figure 2. The sighting points of dolphin Indo-Pacific humpback (40 sighting points) and Irrawaddy (29 sighting points) dolphin.

A pod of Irrawady dolphins sighted in Kuala Larut

Indo-Pacific humpback dolphins spotted in Kuala Sepetang

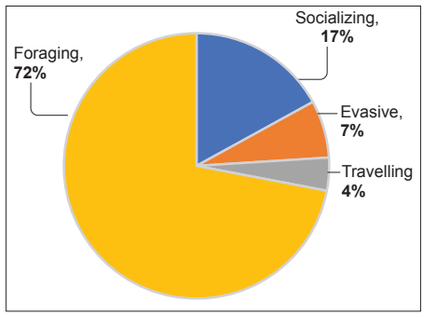

Figure 3: Types of dolphins behavior characterized by foraging, travelling, evasive, and socializing

The significant behaviors of both dolphins in Sg. Matang are indicated in Fig. 3. Foraging was the common behaviour displayed by both dolphins in A and B. This finding suggests that the hotspot areas were a vital foraging site for both dolphins. There were also distinct behaviors of the dolphin species studied. The Indo-Pacific humpback was more sociable with breaching, bow riding, upside-down swimming, and rough playing with each other. On the other hand, the Irrawaddy dolphins were seen as grouped and stayed close until they touched each other. They also tended to spy-hopping when the survey boat was present.

The study on dolphins in Matang was still preliminary. The next study scope may try to answer how human anthropogenic activities affect the habitat of this megafauna, with limitations such as the daytime-only survey and survey during high tide to increase the chance of encountering the dolphins. Studies on genetic trees and stable isotopes for the dolphin are also needed to understand more about them. Nevertheless, Matang waters or Matang Mangrove Forest Reserve area was an essential source for future study and conservation. ■

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Domestication of Giant Freshwater Prawn (*Macrobrachium rosenbergii*)

SAADIAH I*, BALTON M & FRIBM
Fisheries Reasearch Institute Pulau Sayak,
08500 Kota Kuala Muda, Kedah
**saadiah@dof.gov.my*

The Malaysian giant freshwater prawn (GFP) (*Macrobrachium rosenbergii* (de Man, 1879), or udang galah, holds significant cultural and economic importance across Asia and various regions. Over 40 years of cultivation, this species has become a staple in Southeast Asian countries and extends to South Pacific nations, Northern Oceania, and Western Pacific islands.

In Malaysia, the demand for GFP is high for food and recreational purposes. However, the industry faces a critical challenge in insufficient high-quality post larvae (PL) (Banu & Christianus, 2016; Kamarudin & Mhd Ikhwanuddin,2021). The gradual decline in the availability of wild broodstock further worsens this situation, as most GFP PL producers rely heavily on wild broodstock. Domestication of GFP broodstock could overcome this issue. With a systematic breeding program, the sustainability and profitability of the GFP industry could be enhanced.

This article presents an overview of the ongoing R&D program for establishing GFP domesticated broodstock at FRI Pulau Sayak, Kedah. The program primarily involves selective breeding, mainly emphasizing improving genetic traits and other essential factors related to the broodstock's quality. The principal objective of domesticating broodstock is to establish a consistent and reliable supply of high-quality larvae. Maintaining the genetic diversity within the broodstock is vital as a preventive measure against inbreeding, ensuring the long-term health and adaptability of the prawn population. A critical aspect of this process involves vigilant health monitoring and optimizing the broodstock's reproductive performance.

This program was initiated in the 11th Malaysia Plan. In 2016, a base population

Tagging the juvenile prawn with color VIE tag according to the families of the study

Family identification of the coded adults prawn

Harvesting the experimental prawn juveniles that nursed in the B-net

was established for genetic improvement, with an initial population comprising GFP domesticated stocks maintained at FRI Pulau Sayak. Additionally, the program incorporated two wild populations from Sg. Lundu, Sarawak and Sg. Perak, Perak (Hamzah et al., 2022). The program successfully produced the first generation (G1) in 2016, and currently, the broodstocks for the sixth generation (G6) are being prepared for the subsequent generation's production. The data collected from G1 to G6 indicated that the domesticated fry was superior in growth performance and increased productivity. The initial findings were published by Hamzah et al. (2022) with a detailed performance analysis of the domesticated fry, spanning from the base generation to the third generation (G3). As broodstock development is a long-term endeavor, this program will continue in the 12th Malaysia Plan. For the 12th MP, this programme focused on the assessment and verification of the domesticated fry performance in private farms in Baling, Kedah, and Ijok, Perak. The observation obtained so far affirmed the superior quality of the produced PL.

Harvesting adult prawns by farmers

Health management was crucial in this program. Upon arrival at the hatchery, all the GFP broodstock would undergo screening for specific diseases and a particular quarantine period. Furthermore, before being distributed, the PL generated from domesticated broodstock also undergoes screening for a specific disease, particularly M. rosenbergii nodavirus (MrnV).

Although the program has demonstrated positive outcomes, there is a need to further improve the program. Among the improvements is the support for establishing GFP Broodstock Multiplication Centers (BMCs) in other locations, including Perak, Negeri Sembilan, and Johor. This step ensures constant supplies of quality broodstock for industrial players and addresses the pressing demand for high-quality broodstock. Additionally, support from the DOF and cooperation with GFP farmers and other stakeholders is needed to ensure this program's success and long-term viability. ■

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Aquatic Plant Tissue Culture: From Lab to Farm
Acclimatization of *Riccia fluitans* using Open and Close Container Methods

AFZAN MUNTAZIANA MP*, SHARIFAH NURUL NABILA SM, MUHAMMAD MJ, NOR AZURA S & NORHANIZAN S
Fisheries Research Institute Glami Lemi, Jelebu, 71650, Negeri Sembilan
*afzanmuntaziana@dof.gov.my

The Aquatic plant *Riccia fluitans*, is an amphibious liverwort commonly known as crystal wort or floating liverwort (Felix & Sabine, 2020; Wyatt &Davision, 2013), emerges as a compelling subject for tissue culture protocols. With its thalloid morphology and buoyant growth, *R. fluitans* plays vital roles in aquatic ecosystems, including oxygenation, nutrient cycling, and habitat provision. Furthermore, its simple anatomy and reproductive biology make it ideal for tissue culture experimentation.

This study investigated the growth and expansion of *R. fluitans* under various environmental conditions in a tissue culture laboratory and greenhouse at the FRI Glami Lemi, Negeri Sembilan. Firstly, the sterile thallus of *R. fluitans* was sub-cultured in the Murashige and Skoog (MS) medium (Murashige & Skoog, 1962). After two months, the plants were removed from the media and affixed onto stainless steel mesh panels (8 cm × 8 cm), each accommodating 2.5–3 g of *R. fluitans*. Five treatments were conducted: T1 (control): cultivated in a controlled laboratory environment using a closed container; T2: cultivated in a greenhouse using an open container; T3: cultivated in a greenhouse using a closed container; T4: pre-acclimatized for 30 days in a controlled laboratory setting, then cultivated in a greenhouse using an open container; T5: pre-acclimatized for 30 days in a controlled laboratory setting, then cultivated in a greenhouse using a closed container. The study duration was two months.

There were significant ($p < 0.05$) differences in growth rate among the experimental groups. T3 and T5 showed significantly higher growth rates than other treatments. Interestingly, T3 performed better than the laboratory control groups (T1, T4, and T5) regarding growth rate. The exceptional growth rates in T3 and T5 highlighted *R. fluitans* remarkable resilience and adaptability to different environments. Despite the absence of a pre-acclimatization period, these treatments exhibited robust growth, suggesting that *R. fluitans* can flourish when directly transplanted into a farm environment. This finding has profound implications for commercial purposes, as streamlining the cultivation process by bypassing intermediate acclimatization stages enhances efficiency and reduces costs. Moreover, treatments conducted in closed containers (T3 and T5) consistently outperformed those in open containers (T2 and T4). This disparity suggests that enclosing the cultivation environment confers advantages, likely by regulating factors like humidity, temperature, and pest infestations.

Table 1: Generation area of *Riccia fluitans* after two months of treatment (T1–T5)

Treatment	Regeneration Area (cm^2)	Expansion Ratio
T1	9.947 ± 0.7043[a]	2.0623 ± 0.1099[a]
T2	12.5521 ± 2.4588[a]	2.2002 ± 0.2254[a]
T3	23.0833 ± 2.1720[b]	3.2432 ± 0.1371[b]
T4	14.0000 ± 1.4076[a]	2.1745 ± 0.1365[a]
T5	22.6979 ± 1.8019[b]	2.9767 ± 0.2971[b]

Different letters refer to significant differences at the level of $p<0.05$

This study provides valuable insights into cultivating *R. fluitans* under different environmental conditions. The results underscore the plant's adaptability to varying conditions and the critical importance of meticulous cleaning of tissue culture media before transplantation. Contaminants introduced during transplantation compete with aquatic plants for nutrients, potentially threatening the aquatic ecosystem's health. Pathogenic microorganisms can trigger disease outbreaks, leading to crop losses and ecological disruptions.

These findings have practical implications for farmers interested in cultivating *R. fluitans* using tissue culture. The ability to directly plant *R. fluitans* in a farm setting without laborious acclimatization processes in a laboratory holds significant promise. Farmers can bypass the expenses and logistical challenges of maintaining laboratory facilities, reducing operational costs, and streamlining cultivation processes on a larger scale. Further research aimed at optimizing cultivation techniques and understanding the underlying physiological mechanisms could be conducted to support the sustainable aquaculture potentials of *R fluitans*. ■

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Tissue culture of Riccia fluitans after two months in MS medium


Riccia fluitans embedded onto stainless steel mesh panel (8 cm × 8 cm).

Riccia fluitans on the mesh after two months

Yellowfin Tuna:
Sabah Precious Resources

EFFARINA MFA* & SALLEHUDIN J
Fisheries Research Institute Kampung Acheh, 32000 Sitiawan, Perak
**effarina@dof.gov.my*

The Yellowfin tuna (*Thunnus albacares*) is a highly valuable species found in seas across the globe, spanning from 45° N to 45° S of the equator (Collette & Nauen, 1983). The yellowfin tuna—known for its vibrant yellow fins and streamlined body—inhabits Southeast Asia's tropical and subtropical waters. This species is highly sought by industrial and artisanal fisheries in Malaysia, Indonesia, the Philippines, Thailand, and Vietnam. The abundance of yellowfin tuna in Southeast Asian waters has led to targeted fishing efforts to capture this prized species.

Among the critical tuna landing sites in East Malaysia are Kota Kinabalu and Semporna, Sabah since early 2000. The catch contributes significantly to the marine fish landings in East Malaysia, which ranged between 909.44 and 5783.19 metric tons per year from 2010 to 2022. The highest landing was in 2018 (896.98 metric tons) from Kota Kinabalu.

This study examined the length-weight relationships, growth parameters, and mortality rates of yellowfin tuna in Kota Kinabalu and Semporna, Sabah, from 2014 to 2023. The yellowfin tuna samples were collected monthly from handline fishing at both locations. A total of 47,055 yellowfin tuna were sampled from Kota Kinabalu, while 16,060 were sampled from Semporna. Each fish's fork length (FL) was measured to the nearest 0.1 cm, and individual body weights were taken using a mechanical weighing scale. The population parameters, such as growth estimation and mortality, were analysed using the ELEFAN I FiSAT program.

The length frequency analysis of yellowfin tuna showed different size distributions and class modes in each location. In Kota Kinabalu, yellowfin tuna ranged from 20 to 188 cm, with a significant number of individuals (16,576) falling in the 40–50 cm size class. By contrast, in Semporna, yellowfin tuna had a slightly narrower length range of 30–182 cm, with a distinct class mode observed in the 90–99 cm range and 5,908 individuals falling within this size class. These findings suggest that the size structure of yellowfin tuna populations varies between the two locations, possibly due to environmental conditions, fishing pressure, and habitat preferences.

Length frequency data was used to calculate the von Bertalanffy growth rate (K) and the asymptotic length ($L\infty$) by model progression analysis using the program ELEFAN I within the ELEFAN I FiSAT program (Gayanilo et al., 2005). The analysis of the growth parameter estimation for yellowfin tuna revealed that the maximum length ($L\infty$) was consistent in both areas, measuring 189 cm. Yellowfin tuna in the Kota Kinabalu area has a growth coefficient (K) of 0.33 $year^{-1}$ and a performance index (Ø') 4.03. By contrast, the Semporna area has a higher growth coefficient (K) of 0.93 $year^{-1}$ and a performance index (Ø') of 4.49.

Table 1 lists the mortality and exploitation rates for yellowfin tuna in Kota Kinabalu in the present and previous studies. The results revealed significant differences in the length–weight relationships between the two locations. Specifically, Kota Kinabalu exhibited positive allometric growth, while

Semporna showed negative allometric growth. Furthermore, the growth parameters varied between the two locations, with Semporna having a notably higher growth coefficient (K) than Kota Kinabalu. Notably, the mortality and exploitation rates also differed among regions, reflecting different levels of fishing pressure and environmental conditions. Overall, these findings emphasize the complex dynamics of yellowfin tuna populations across various geographical areas and highlight the necessity of tailored management strategies for ensuring sustainable fisheries

Table 1: Mortality and exploitation rate of yellowfin tuna from several areas

Location	Z	M	F	E	References
Kota Kinabalu, Sabah	1.2	0.53	0.67	0.56	Present study
Semporna, Sabah	5.52	1.22	4.30	0.78	Present study
Banda Sea, Indonesia	1.04	0.55	0.49	0.55	Amri et al. (2021)
Indian Ocean	2.32	0.69	1.63	0.70	Ghofar et al. (2021)
Banda Sea, Indonesia	1.47	0.49	0.98	0.67	Haruna et al. (2018)

These findings provide valuable insights into yellowfin tuna's mortality and exploitation rates across different geographical areas, essential for informing sustainable fisheries management strategies and conservation efforts. Monitoring and assessing yellowfin tuna populations in these regions is necessary to ensure long-term sustainability. This finding could involve ongoing research to understand better the factors that drive the observed differences in growth patterns and mortality rates. It is also essential to implement adaptive management approaches considering the regional variability in fishing pressure, environmental conditions, and population dynamics. ■

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Water Quality Profiles of Patin (*Pangasius* sp.) Cage-Culture Areas along the Sungai Pahang

RIMATULHANA R*, MUHAMMAD IZZUAN R, MUHAMAD SYAFIQ IZZUDDIN AH
National Fish Health Research Centre, 11960 Batu Maung, Penang
*rimatulhana@dof.gov.my

The state of Pahang contributed substantially to aquaculture production in Malaysia, with 13,143.29 tonnes of freshwater fish (53.37% of aquaculture production) in 2022 (DOF 2023). Sg. Pahang is a prominent area for aquaculture, with Pangasius (*Pangasionodon hypophthalmus*) or Patin hitam as the major species cultured (63% of Pahang's total freshwater fish production). However, farmers in Sg. Pahang frequently experienced fish mortality and substantial economic losses, primarily due to disease, environmental changes, and industrial discharge (Roselan, 2016), with increasing severity and losses, particularly during March and April (Latib, 2018; Rosedi, 2021).

Because of this, a study was initiated to comprehend and manage this issue. Under the 12th Malaysia Plan, a project (under P21300040170502: Research and Development in Aquaculture Fish Health Program) was carried out to collect data on mortality cases and associated factors to recommend mitigation measures. This article presents part of the results focussing on the water quality of Sg. Pahang over two consecutive years as water quality is one the most crucial factors affecting fish growth, reproduction, and production. Ten locations were selected along Sg. Pahang comprises minimum (6 cage units), moderate, and dense farming areas. These locations are approximately 40 km apart (except for the first and second sites, approximately 80 km upstream from Kuala Tembeling. Monthly sampling was conducted from January 2022 to December 2023 to determine the physical (*in situ*) and chemical water quality parameters.

Table 1: Sampling locations selected in this study

District	Sampling location	
	2022	2023
Lipis	1. Kg. Beralas	1. Kg. Beralas
Jerantut	2. Kg. Pagi	2. Kg. Bantal
	3. Kg. Lada	3. Kg. Lada
Temerloh	4. Kg. Tg. Kubu	4. Kg. Tg Kubu
	5. Kg. Kerdau	5. Kg. Kerdau
Bera	6. Kg. Paya Panjang	6. Kg. Paya Panjang
Maran	7. Kg. Nyak	7. Kg. Pengkalan Balai
Pekan	8. Kg. Kuala Chini	8. Kg. Kuala Chini
	9. Kg. Terlang	9. Kg. Terlang
	10. Kg. Medang	10. Kg. Medang

In general, the water quality readings at Sg. Pahang for 2022 was satisfactory for aquaculture endeavours, with critical indicators (dissolved oxygen (DO), pH, temperature, and ammonia) consistently showing acceptable levels throughout the year. Nevertheless, water quality may deteriorate under extreme weather conditions or heavy rainfall. Meanwhile, a relatively high ferum concentration exceeded the aquaculture's recommended limit was recorded. This finding could be attributed to long-standing mining activities in this area. High ferum content may pose a potential threat to fish respiratory systems.

In 2023, changes in water quality parameters were observed at several locations (Jerantut and Maran) where there were no ongoing fish farming activities. Two other locations, Kg. Medang (Pekan) and Kg. Paya Panjang (Bera), had also experienced changes for the same reason (Table 1). In 2023, readings for dissolved oxygen, pH, total suspended solids (TSS), and ammonia were better than in 2022. Generally, there was an increase in several parameters compared to 2022, including temperature, DO, pH, alkalinity, phosphate, and ferum. Other parameters either decreased or remained the same. The overall data was still being analysed. We noted a significant increase in river water temperature ($p<0.05$) in Sg. Pahang. This finding has adversely affected the health of tilapia and catfish, leading to mortalities. Moreover, ferum concentration showed an increase compared to the previous year. This is likely due to the prolonged hot weather conditions, which caused ions and particles to concentrate in the water because of reduced water volume (Table 2). The TSS and ammonia levels were the main factors influencing the mortality rate of catfish in Sg. Pahang, both of which correlated directly with rainfall distribution. The interaction of environmental factors such as ferum content, oxygen levels, pH, and water quality, along with management issues like poor nutrition and overcrowding, may heighten stress in cultured fish, making them more prone to disease outbreaks.

Table 2: Ranges of main water quality parameters recorded at Sg. Pahang fish cages in 2022 and 2023

Water quality parameters	2022	2023	Optimum range
Temperature (°C)	27.7 ± 1.27	28.3 ± 1.60	25-32
Dissolved oxygen (DO) (mg/L)	5.05 ± 1.26	6.37 ± 0.96	>5
pH	6.89 ± 0.37	7.1 ± 0.35	6.5-8.5
Alkalinity (mg/L)	22 ± 7.04	31 ± 8.65	>20
Ammonia nitrogen (NH^3-N) (mg/L)	0.98 ± 0.58	0.89 ± 0.53	0.7-1
Salinity (ppt)	0.02 ± 0.01	0.02 ± 0.01	<2
Nitrate (NO^3-N) (mg/L)	0.9 ± 1.17	0.8 ± 0.69	<7
Nitrite (NO^2-N) (mg/L)	0.011 ± 0.02	0.005 ± 0.01	<0.1
Total suspended solids (TSS) (mg/L)	137 ± 94.35	111 ± 80	25-150
Total dissolved solids (TDS) (mg/L)	33.66 ± 6.77	36 ± 11.47	400
Phosphate (mg/L)	0.20 ± 0.17	0.26 ± 0.14	0.005-0.2
Ferum (mg/L)	1.37 ± 0.69	1.97 ± 1.91	0.35-1.0

In summary, the water quality of Sg. Pahang is generally conducive to patin culture. However, during extreme weather events like droughts or floods, there may be fluctuations in water quality, potentially impacting the well-being of farmed fish and causing disease and mortality. ■

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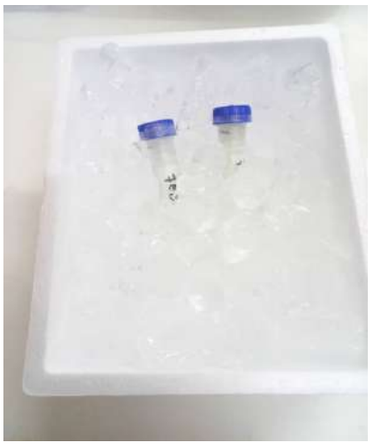
Development of Effective Giant Grouper (*Epinephelus lanceolatus*) Sperm Transportation Method

MOHD KHAIRUDIN M* & SUFIAN M

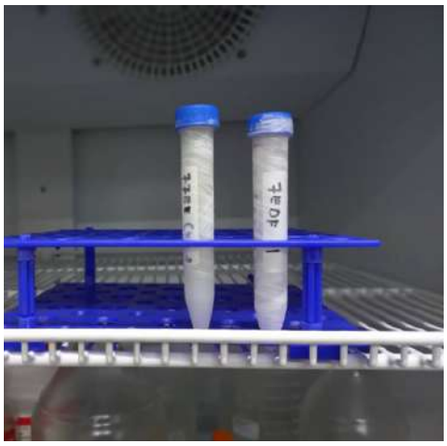
Fisheries Research Institute Tanjung Demong, 22200 Besut Terengganu

**mohdkhairudin@dof.gov.my*

Hybrid groupers are highly popular among breeders in Malaysia and Southeast Asia. The hybrid grouper is the product of the crossing between a giant grouper (*Epinephelus lanceolatus*) male and a tiger grouper (*Epinephelus fuscoguttatus*) female. Groupers are hermaphrodite fish species where sex changes from female to male occur as they mature. This characteristic has posed challenges for breeders in synchronizing the availability of gametes from both sexes for fertilization. Plus, the quality of fresh sperm declines rapidly unless it is diluted and chilled.



Sperm delivered in crushed-ice



Sperm stored in a chiller

Thus, a study was initiated in the 12th Malaysia Plan to develop an effective sperm transportation method. From the literature, sperm cooling and freezing have been demonstrated to assist in preserving the motility of sperm by reducing the metabolic activity of sperm and encouraging them to turn into hibernation mode. To maintain the quality of sperm for a long duration after being stripped out from the broodstock body, fresh sperm needs to be placed in an extender solution. Each species has a specific extender solution. The best extender solution for giant grouper is artificial seminal plasma (ASP) that contains sodium chloride (135 mM), potassium chloride (2 mM), magnesium chloride (2.3 mM), calcium chloride (1.3 mM), sodium bicarbonate (20 mM), Hepes (20 mM). The optimal ratio for diluting siant grouper sperm with an ASP extender is 1:3. Firstly, the giant grouper sperm should be free from contaminants upon being stripped from broodstock. Then, the motility of fresh sperm will be determined using computer-assisted-semen-analysis (CASA). Then, the sperm were diluted with an ASP extender. Only semen with more than 90% motility would be used for dilution with ASP. Subsequently, the diluted sperm was covered in crushed ice and stored in a polystyrene box. This sperm will be delivered to farmers across Malaysia and may survive for up to 5 days when stored at 4˚C. Within five days, the farmers can use the sperm for fertilization with tiger grouper eggs. In 2023, FRI Tanjung Demong transported seven shipments of giant grouper sperm (total: 360 mL) to various locations throughout Malaysia.

In conclusion, this technique is practical and viable for transporting giant grouper sperm. It minimizes time and costs, as the farmers only need female tiger grouper broodstock for breeding. Hopefully, this method will facilitate marine fish breeding activities and ultimately enhance marine fish production in Malaysia. ■

Water Quality Assessment for Mollusc Farming in The Sungai Merbok Estuary

ROZIAWATI MR*, MOHD NOR AZMAN A, MASAZURAH AR, NAJIHAH M, MUHAMMAD FAROUK H & INTAN NURLEMSHA B
Fisheries Research Institute, 11960, Batu Maung, Penang
**roziawati@dof.gov.my*

Sungai (Sg.) Merbok, Kedah is the central feature of Sg. Merbok Mangrove Forest Reserve was established in 1951. It is extensively used for aquaculture, including fish, shrimp, and oysters farming and harvesting wild molluscs. Despite its importance, limited studies have been conducted to assess the pollution levels in this crucial aquatic body since Sg. Merbok is exposed to many anthropogenic activities, including agriculture, manufacturing, and residential. Anthropogenic activities have been demonstrated to induce high nutrient influxes (Hodgkiss & Lu, 2004) and low oxygen levels (2-3 mg/L) that may lead to hypoxia (Saha et al., 2022), which is perilous for caged fish (Alosairi et al., 2021). These circumstances may directly impact the growth, survival, and reproduction of aquatic species. Moreover, bacterial proliferation and harmful algal blooms also thrive in this environment, posing risks to aquatic life and humans (Chintagari et al., 2017).

Water quality parameters in Sg. Merbok has been shown to exceed the acceptable limit of the Marine Water Quality Standard (MWQS) for Malaysia (Fatema et al., 2014). Given its significance in mollusk farming and aquaculture activity, there is a need for the latest assessment. Under the 12th Malaysia Plan, FRI Batu Maung and the Japan International Research Center for Agricultural Sciences initiated a study in 2022 and is still on going to i) identify and quantify the presence of potentially harmful microalgae water samples, ii) determine the microbial contents in oysters and water samples, iii) detect the presence of Paralytic Shellfish Poisoning (PSP) toxin in oyster and iv) evaluate the physicochemical properties of water samples. Four sampling stations were selected for water quality, harmful algae, faecal bacteria, biotoxin, and heavy metal analysis (Fig. 1), whereas eleven stations were chosen for microplastic assessment (Fig. 2).

In situ measurements of water quality parameters such as chlorophyll a, pH, temperature, dissolved oxygen (DO), turbidity, total suspended solids, and salinity were recorded. Water samples and shellfish were returned to the laboratory to analyze nutrients (nitrate, ammonia, phosphate), heavy metals, microbial, microplastic, and PSP toxin.

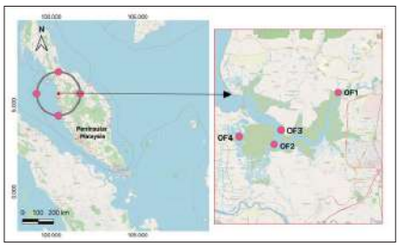


Figure 1: Sampling locations selected in this study

Potentially toxic microalgae such as *Alexandrium* sp., *Dinophysis caudata*, *Dinophysis novergica*, *Pseudo-nitzschia,* and *Prorocentrum micans* were detected at all sampling stations throughout the sampling period but at low cell densities except in April and May 2022 for *Alexandrium* sp. A low level of PSP toxin was recorded in oysters (0.29-4.39 µg eq. STX/100g), way below the safety level of 80 µg eq. STX/100g.

Nitrate, DO, phosphate, and ammonia concentrations had exceeded the MWQS, indicating poor water quality in the Sungai Merbok estuary (Table 1). The physicochemical characteristics showed the water temperature, salinity, and pH levels ranging from 27.00 °C to 32.70 °C, 4.30 to 30.01 ppt, and 5.68 to 8.54, respectively.

Table 1: The range of the seawater physicochemical parameters recorded in Sg. Merbok, Kedah

Stations	St. 1	St. 2	St. 3	St. 4
DO (mg L^{-1})	0.65-11.22	1.91-16.30	2.05-17.56	1.91-7.19
Nitrate (mg L^{-1})	0.02-0.61	0.01-1.01	0.01-0.70	0.01-0.44
Ammonia (mg L^{-1})	0.01-1.28	0.0-0.51	0.0-0.58	0.0-0.16
Phosphate (mg L^{-1})	0.07-0.41	0.03-0.92	0.02-0.36	0.05-0.25

The average coliform and fecal coliform concentrations in water samples from the four stations were 156/100 mL to 341/100 mL and 12/100 mL to 20/100 'mL, respectively. Overall, total coliform and fecal coliform concentrations in water samples from all sampling stations were relatively high and classified as Class IIA according to the National Water Quality Standards for Malaysia. Oyster samples were mostly from Class B (less than 4,600 total quantity of E. coli shells/100 g or less than 6,000 fecal coliform/100 g), indicating suitability for consumption only after thorough cooking. *Salmonella* sp., *Vibrio cholerae*, *V. parahaemolyticus,* and Hepatitis A virus were not detected in oyster samples from any stations. Meanwhile, low concentrations of heavy metals (magnesium, aluminum, manganese, iron, and selenium) were detected in the water samples, which comply with the regulatory guidelines for human health and the ecosystem.

Regarding microplastic analysis, the highest microplastic particles were recorded at stations SM5, SM11, and SM9, particularly in areas with active aquaculture activities (Figure 2). ■

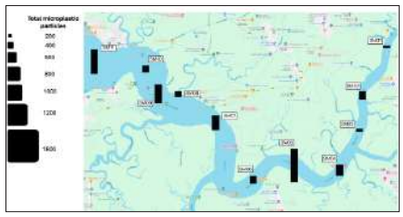


Figure 2: Total microplastic particles from eleven sampling stations in Sungai Merbok, Kedah

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Rapid Setting Cement-Based Mortars as a Substrate for Coral Cultivation

MS ISMAIL
National Marine Park Research Centre, Fisheries Research Institute,
11960, Batu Maung, Penang
*saupi@dof.gov.my

Technical field of invention

This invention was registered as copyright in 2021 (LY2021P04201). The invention relates to a method and device for cultivating small-sized coral fragments or nubbins using rapid-setting-cement-based mortars as substrates for coral propagation in nurseries and natural habitats. The method comprises securing a coral fragment/nubbin to the substrate by mounting the fragment/nubbin onto the cement-based mortar. The invention is of value to educational, scientific research, and commercial aquarium industry purposes.

Justification of the invention

The worldwide decline in coral coverage due to climate change and associated coral bleaching, overfishing, coastal pollution, and other direct human impacts such as destructive fishing and illegal coral collection is a significant concern, justifying the need to cultivate corals for restoration and rehabilitation purposes (Ismail & Hassan, 2005; Ismail & Goeden, 2022).

Various coral conservation and restoration methods were proposed, including *in-situ* (in the sea) and *ex-situ* nurseries (on land) (Ismail et al., 2022). While *in situ* nurseries are increasingly used as reef restoration tools, *ex-situ* nurseries have not been utilized extensively except by those culturing corals for the aquarium trade (Ismail et al., 2022).

In Malaysia, most coral cultivation was conducted through the *in-situ* method (Ismail et al., 2014), where small coral fragments are cultured in mid-water or benthic nurseries until large enough to survive. However, small pieces do not survive well *in situ* but can thrive and grow in *ex situ*. One of the advantages of the *ex-situ* culture is that the nurseries can be initiated by copy editors, most of which have little or no impact on the donor coral population. Parameter's indicative of coral health, such as survivorship, growth, self-attachment times, and bleaching rates, commonly monitored in *in situ* nurseries, are valuable and pertinent when managing *ex-situ* nurseries (Ng et al., 2012). However, the information on the methodology for *ex-situ* coral nurseries to house asexually propagated corals, especially in Malaysia, remains insufficient (Ismail et al., 2014). Thus, there is a need to develop a method that effectively gauges the effort required for asexual coral propagation for reef restoration and coral farming.

This invention aims to provide an effective, practical, and inexpensive method and device for the propagation and establishment of new coral colonies. The objectives are to assess the feasibility of producing transplantable coral colonies by mounting the coral to rapidly setting cement-based mortars and determining the growth and survival rates of corals reared on those substrates.

Summary of the Invention

This invention presents a new technique and methodology for securing coral fragments to a substrate for coral cultivation. The chosen product is a rapid-setting-cement-based mortar, which is non-flammable, chloride-free, approved for use in public water supplies, and chemically stable under marine conditions throughout the invention. This method's suitability was assessed using four (4) scleractinian coral species, i.e., *Favites abdita*, *Galaxea fascicularis*, *Lobophyllia corymbosa,* and *Turbinaria peltata*. Survival and growth of coral fragments were recorded monthly. After a year, the survival rates of the fragments were 88.89%, 93.75%, 100%, and 90% for *F. abdita*, *G. fascicularis*, *L. corymbosa,* and *T. peltata,* respectively (Table 1). The relatively high survival rates suggest these corals could be reared on the substrates. The method of the invention is effective in securing the coral fragments and requires minimal space to produce larger coral colonies. In conclusion, this study recommends using a rapid setting cement-based mortar as a suitable substrate for coral propagation of different coral species.

Table 1: Survival and growth of coral fragments in the nursery

Species	No (n)	Survival (%) *	Mean initial size ($cm^2 \pm sd$)	Mean end size ($cm^2 \pm sd$)	Average Increase (%)
F. abdita	18	88.89 (16)	27.30±11.39	44.20±18.81	61.9
G. fascicularis	13	93.75 (12)	18.80±4.44	37.60±7.37	100
L. corymbosa	36	100 (36)	27.10±10.67	48.30±13.05	78.2
T. peltata	10	90 (9)	16.33±7.84	25.44±7.68	55.8

*Number of fragments (n) after one year is given in parentheses.

Findings

The asexual propagation of coral was described by Ismail et al. (2022). The coral fragments were cut from the mother colony (Fig. 1). A rapid-setting-cement-based mortar was poured onto a cement, paper, or plastic cup (diameter of

4–6 cm) as a substrate. Then, clean waters were added with water: powder ratio of 1:3 (by volume) and appropriately mixed using a spatula, spoon, or stirrer (Fig.2). The minimum thickness of the substrate was approximately 2 cm. The solidification process took less than a minute. The average size of coral fragments was approximately 5 cm. The coral fragment's base or bottom side was dipped into the substrate, with the length of the submerged part less than 2 cm, right before it became solid. Later, the cup was immersed in seawater. After at least half an hour, the paper or plastic cup was carefully taken out, leaving the hardened substrate with a fragmented coral mounted on it.

The results of this study indicated that it was feasible to asexually propagate certain scleractinian coral species and achieved appreciable survivorship and growth on the rapid-setting-cement-based substrates. No coral fragments exhibited any bleaching at all during the entire duration of the study. Almost all nubbins survived over the study periods, indicating that all transplanted species were adapted to the aforementioned substrates.

The self-attachment exhibited by the coral species in this study indicated that the substrate was conducive to the growth of scleractinian corals. These outcomes have positive implications for the potential to culture those four corals and possibly other genera from small fragments (Ismail & Hassan, 2005; Ismail & Peng, 2016). Thus, the rapid setting cement-based mortar has been proven not to hinder the growth of corals and may be chosen as an artificial substrate for coral propagation. The transplanted corals, mounted on the aforementioned substrates, can be cultivated *in situ* using a patented apparatus for coral cultivation (patent filing no: PI2019007467, dated 12 May 2020), as shown in Fig. 3. This simple, inexpensive, practical, and environmental-friendly coral frame, My Coral Tripod, is suitable for in situ coral farming and conservation in Malaysia (Ismail et al., 2023). ■

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Figure 1: The coral fragments were cut from the mother colony

Figure 2: Preparation of cement-based mortar

Figure 3: The transplanted corals on cement based mortar on coral tripod

FRI@MAHA 2024