

Vol.27, 2025



MALAYSIAN FISHERIES JOURNAL

A Journal of the Fisheries Research Institute,
Department of Fisheries, Malaysia

Malaysian Fisheries Journal
Volume 27 (2025)

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Published by

Fisheries Research Institute

11960, Batu Maung, Pulau Pinang

MALAYSIA

ISSN 1511-7286

Editor's Note

Welcome to the latest edition of the Malaysian Fisheries Journal (MFJ) Volume 27 (2025), an annual publication by the Fisheries Research Institute (FRI), Department of Fisheries Malaysia. MFJ is a platform built to empower our researchers, technical staff and field experts to share their discoveries, innovations and insights. We also warmly invite contributions from universities and agencies researchers with a passion for fisheries and aquaculture, as we believe that collaboration across institutions and disciplines is key to solving today's challenges. In this issue, I am excited to welcome our new generation of editors, coming from a variety of disciplines, which bring fresh energy and insight that will sharpen our review process and deepen our appreciation of the research submitted. We're excited to grow together with their contributions. Also, we're also excited to announce that MFJ now has its own tab on the FRI website, making it easier than ever to access and contribute: <https://fri.dof.gov.my/en/malaysian-fisheries-journal-mfj-2/>

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Preliminary investigation on the presence of invasive alien species in Sungai Perai, Penang, Malaysia

MOHAMAD SUFIYAN SALMI¹, WAN NORHANA MD NOORDIN², SITI NORITA MOHAMAD^{1*}, HASLAWATI BAHARUDDIN¹

¹Fisheries Research Institute, FRI Glami Lemi, 71650 Titi, Negeri Sembilan, Malaysia

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

*Corresponding author: ctnorita@dof.gov.my

Abstract: The issue of Perai River (Sg. Perai) has come to attention in 2017 with allegations from the local fisherman about the dwindling catches of freshwater prawn from this river due to alien fish species. Thus, a basic study was implemented to assess the composition of fish species in Sg. Perai. Two sampling activities were carried out at 12 sampling stations along the river. Four pieces of equipments were used to catch aquatic life namely nets, cast nets, splints and fish traps according to in-house procedures established by the Fisheries Research Institute Glami Lemi, Negeri Sembilan. A total of 21 fish species comprising 16 families were recorded. Parastacidae is the dominant family in terms of species, representing 45.40%, followed by Palaemonidae (31.28%) and Gecarcinucidae (13.49%). The other families only constitute about 0.09-2.59% of the total species recorded in this study. Out of 21 fish species, 14 were native, while seven (*Cherax quadricarinatus*, *Hypostomus plecostomus*, *Clarias gariepinus*, *Oreochromis niloticus*, *Pangasius* sp., *Barbonymus gonionotus* and *Amphilophus citrinellus*) were alien. *C. quadricarinatus* was the most commonly encountered alien species in Sg. Perai. *C. quadricarinatus* recorded the highest number of catches and percentage, n=508 (45.4%), followed by freshwater prawn (*Macrobrachium rosenbergii*) (n=350, 31.3%) and rice crab (n=151, 13.5%). The most frequently found sizes are between 11-20 g (n=103), 21-30 g (n=96) and 31-40 g (n=71). Female *C. quadricarinatus* were commonly encountered (n=351) compared to males (n=146) with 11 (3.1%) of them being spawning females. The dominance of *C. quadricarinatus* in Sg. Perai ecosystem is significant and should alert the stakeholders to initiate comprehensive efforts in Sg. Perai ecosystem management, conservation and economic stability.

Keywords: *Macrobrachium rosenbergii*, *Cherax quadricarinatus*, diversity, pollution

Abstrak: Isu ancaman spesies ikan asing di Sungai Perai (Sg. Perai) menjadi perhatian pada tahun 2017 dengan dakwaan nelayan tempatan mengenai pengurangan tangkapan udang air tawar dari sungai disebabkan perkara ini. Justeru, satu kajian awal telah dilaksanakan untuk menilai komposisi spesies ikan di Sg. Perai. Dua aktiviti persampelan telah dijalankan di 12 stesen persampelan di sepanjang sungai. Empat peralatan telah digunakan untuk menangkap hidupan akuatik iaitu pukat, pukat tuang, belat dan bubu naga mengikut prosedur yang dibangunkan oleh Institut Penyelidikan Perikanan Glami Lemi, Negeri Sembilan. Sebanyak 21 spesies ikan yang terdiri daripada 16 famili telah direkodkan. Famili Parastacidae mendominasi dari segi spesies mewakili 45.40% jumlah tangkapan, diikuti oleh Palaemonidae (31.28%) dan Gecarcinucidae (13.49%). Keluarga lain hanya membentuk kira-kira 0.09-2.59% daripada jumlah spesies yang direkodkan dalam kajian ini. Daripada 21 spesies ikan, 14 adalah ikan asli, manakala tujuh (*Cherax quadricarinatus*, *Hypostomus plecostomus*, *Clarias gariepinus*, *Oreochromis niloticus*, *Pangasius* sp., *Barbonymus gonionotus* dan *Amphilophus citrinellus*) adalah ikan asing. *C. quadricarinatus* ialah spesies asing yang paling kerap ditemui di Sg. Perai. *C. quadricarinatus* mencatatkan jumlah tangkapan dan peratusan tertinggi, n=508 (45.4%), diikuti udang air tawar (*Macrobrachium rosenbergii*) (n=350, 31.3%) dan ketam padi (n=151, 13.5%).

Saiz yang paling kerap ditemui ialah antara 11-20 g (n=103), 21-30 g (n=96) dan 31-40 g (n=71). *C. quadricarinatus* betina lebih kerap ditemui (n=351) berbanding jantan (n=146). Sejumlah 11 (3.1%) daripadanya betina yang ditemui mempunyai telur. Dominasi *C. quadricarinatus* di ekosistem Sg. Perai adalah signifikan dan perkara ini perlu dicakna pihak berkepentingan dalam memulakan usaha komprehensif dalam pengurusan, konservasi dan kestabilan ekonomi Sg. Perai.

Introduction

Perai/Prai River or Sungai Perai/Prai (after this refer to as Sg. Perai) basin is located at 5°17'5°33"N latitude and 100°22'-100°38'E longitude in the central part of Seberang Perai, in the state of Penang, north of Peninsular Malaysia, facing Penang Island. Formerly, this river served as a physical border between Seberang Perai (Province Wellesley) under the British-administration and Kedah (Siamese vassal state). The river got its name from the Siamese/Thai word plai (ปลาย) which means "the end". Sg. Perai, hence marked the southernmost limit of Siamese influence. The British named the river "Prye". Although the border between Seberang Perai, Penang and Kedah were shifted north to Sungai Muda later, the name of the river remained.

Sg. Perai starts from the confluence of three big tributaries i.e., Sg. Kulim, Sg. Jarak and Sg. Kreh at the base of the mountain in Kedah and flows into the Straits of Malacca, meandering south-westward (JICA, 1988). Sg. Perai now serves as the natural border between Seberang Perai Utara and Seberang Perai Tengah. The topography is generally flat and lowers coastward. It is the longest river in Penang, with a length of more than 20 kilometres and has a total catchment area of approximately 497.4 square kilometres of which constitutes the largest water geographical region in Penang. Originally, the land along the river was mainly for agriculture with paddy fields in the upstream and midstream while coconut and rubber estates dominates the lower parts. However, starting from the 80s, the lower reach of Sg. Perai has been developed as an industrial and housing area. At present, Sg. Perai passes through residential, industrial and agricultural areas with human activities.

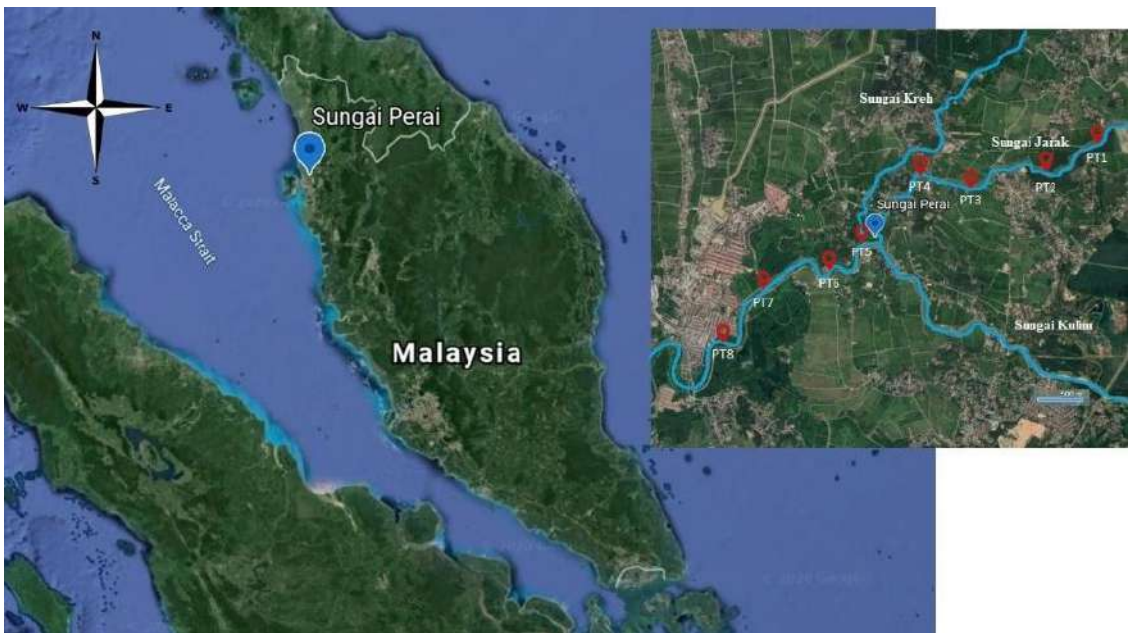


Figure 1. Sg. Perai (highlighted in blue) in Google maps.

The riverine area of Sg. Perai holds various treasures of diverse flora and fauna. In 2017, there was a report in a local newspaper on the negative impacts of alien species such as African catfish and others to the native fish species resources in several rivers in Malaysia, including Sg. Perai (Berita Harian March 12, 2017), which has interfered with fishermen's income. According to IUCN Issues Brief (2021), an alien species refers to an animal, plant or other organism that is introduced by humans, either intentionally or accidentally, into places outside its natural range. Some alien species are classified as 'invasive' when they become established and negatively impact native biodiversity, as well as the ecosystem services on which humans depend. Examples of aquatic alien species in Malaysia are marbled crayfish, red claw crayfish, peacock bass, Pacific oyster, algae suckermouth catfish, Asian red tail catfish, red-striped earth-eater, flowerhorn, freshwater stingray, paddlefish and snowflake coral (NCIAS, 2018).

Sg. Perai is known as one of the niches for the giant freshwater prawn and famous among local fishermen and anglers. For the last few years, the fishermen Sg. Perai have experienced a decline in their catch. Since there is not much information about the composition of fish in Sg. Perai, the Fisheries Research Institute (FRI) Glami Lemi, Jelebu, Negeri Sembilan has taken the initiative to investigate this allegation. A brief study was conducted between April and July 2018 to determine the distribution of aquatic life species in Sg. Perai, especially the composition of invasive alien fish species here. This paper presents the results obtained from the investigation.

Materials and Methods

Study area

A total of 12 sampling stations have been identified along the river (Figure 2). The coordinates of each sampling station and general descriptions were summarized in Table 1. All the sampling sites were accessible via road. In general, the sampling stations constituted Sg. Perai main river (PT5, PT6, PT10, PT 11, PT12), Sg. Jarak (PT1, PT2, PT3, PT4), Sg. Air Hitam (PT7) and Sg. Kulim (PT8 and PT9) tributaries.

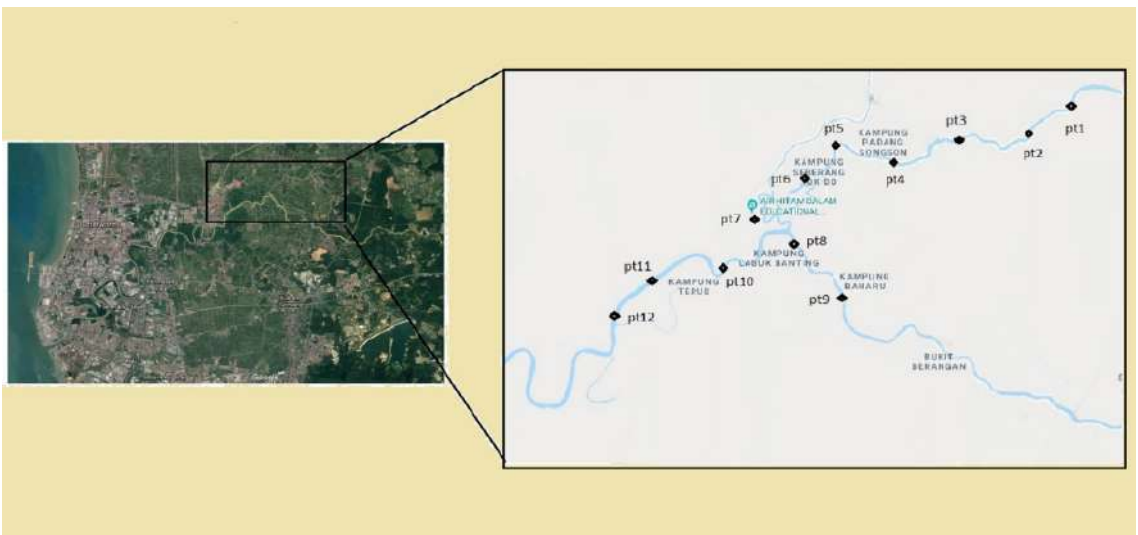


Figure 2. Location of Sg. Perai in northern region of Peninsular Malaysia and the sampling stations involved in this study.

Table 1. Details of sampling stations selected and brief description.

Sampling stations	Coordinate	Streambed type/site description
PT1	N 05° 27' 30.9" E 100° 27' 46.4"	Sg. Jarak tributary. Muddy riverbanks and sandy riverbeds
PT2	N 05° 27' 30.9" E 100° 27' 46.4"	Sg. Jarak tributary. Muddy riverbanks and sandy riverbeds
PT3	N 05° 27' 30.8" E 100° 27' 52.9"	Sg. Jarak tributary. Muddy riverbanks and sandy riverbeds
PT4	N 05° 27' 30.9" E 100° 27' 46.4"	Sg. Jarak tributary. Muddy riverbanks and sandy riverbeds
PT5	N 05° 27' 48.6" E 100° 28' 05.9"	Sg. Perai main river. Muddy riverbanks and riverbeds
PT6	N 05° 27' 38.2" E 100° 28' 52.0"	Sg. Perai main river. Muddy riverbanks and riverbeds
PT7	N 5°27'14.28" E 100°27'10.59"	Sg. Air Hitam tributary (Air Hitam Recreational Forest)
PT8	N 05° 26' 34.2" E 100° 27' 45.2"	Sg. Kulim tributary. Sandy river
PT9	N 05° 26' 46.1" E 100° 27' 34.4"	Sg. Kulim tributary. Sandy river
PT10	N 5°26'45.30" E 100°26'56.36"	Sg. Perai main river. Muddy river
PT11	N 5°26'39.45" E 100°26'27.94"	Sg. Perai main river. Muddy river
PT12	N 5°26'29.61" E 100°26'16.20"	Sg. Perai main river. Muddy river

Sampling activities

Before fish sampling activities were carried out, several *in situ* water quality parameters were recorded. At each station, a multi-probe water quality (YSI 556) was used to record dissolved oxygen (DO) (mg/L), pH, temperature (°C), whereas ammonia (NH₃-N) (mg/L), ferum (Fe) (mg/L), phosphate (PO₄) (mg/L) and alkalinity (mg/L) were quantified using HACH field kit. The readings were compared to the National Water Quality Index (DOE, 2020). The ammoniacal nitrogen can be toxic, while PO₄ is a non-toxic component that could affect the productivity of the aquatic system when it appears in large concentrations.

Table 2. The classes of water under the National Water Quality Standards and Water Quality Index (DOE, 2020) in accordance with the water quality parameters collected in this study.

	Class 1	Class IIA/IIB	Class III	Class IV	Class V
Dissolved Oxygen (mg/L)	>7	5-7	3-5	1-3	<1
pH	6.5-8.5	6-9	5-9	5-9	-
Temperature (°C)	-	Normal ± 2	Normal ± 2	-	-
Ammonia Nitrogen (mg/L)	<0.1	0.3	0.9	2.7	>2.7
Phosphate	Absent	0.2	0.1	-	-
Ferum (mg/L)	Absent	1	1		

Sampling was done twice, in April and July 2018 to assess the distribution of species present in Sg. Perai. To ensure maximum catch, four equipments were used to catch aquatic life from Sg. Perai, namely nets (mesh sizes of 2.5, 3.8, 5.1, 7.6 and 10.2 cm), cast net (jala), splints (belat) and fish trap (kambang/bubu naga) (Figure 3). Each fish specimen collected was measured for total length (TL) and wet weight (g) using a weighing machine, identified at species level using standard taxonomic references (Kottelat, 2013) and photographed for further reference. The number of individuals per species was counted. Data collected were calculated and analyzed for diversity, evenness and richness indexes based on Shannon and Weaver (1963), Pielou (1969) and Margalef (1958).



Figure 3. Four sampling equipment's used to catch the aquatic organisms i.e. (A) net, (B) cast net; (C) fish trap and (D) splint.

Results and Discussion

Water quality

In general, *in-situ* water quality parameters readings for 12 sampling stations were presented in Table 2. The physico-chemical water quality readings were between 3.50-7.34 mg/L for DO, 5.94-6.52 for pH and 28.27-30.74°C for temperature. Sampling stations along Sg. Perai main river recorded low DO readings, particularly at station PT6 (3.50±0.07 mg/L), PT5 (3.72±0.11 mg/L), PT10 (3.86±0.04 mg/L) and PT11 (3.98±0.11 mg/L) thus qualifying it to falls in Class III category in National Water Quality Index (DOE, 2020). This is not surprising as other reports also disclosed that Sg. Perai basin

is polluted and not suitable to be extracted for domestic consumption (Anon, 2020; Mohamad Faudzi et al., 2023). Ammonia and phosphate contents were very high, particularly downstream of Sg. Perai (PT10, PT 11 and PT12) with readings between 4.00-4.46 mg/L and 3.81-3.95 mg/L. Sg. Perai was also reported to be contaminated with heavy metals such as mercury (Hg), aluminium (Al), iron (Fe) and manganese (Mn) from the industrial and domestic waste discharge into the river (Sekhar, 2022). Ferum content was more than 1.0 mg/L in 3 (PT10, PT 11 and PT12) out of 5 sampling stations along Sg. Perai.

Table 2. The average of *in situ* reading of selected physico-chemicals water quality parameters by sampling stations during the study period.

Sampling Point	Dissolved Oxygen (mg/L)	Temperature (°C)	pH	Ammonia (mg/L)	Ferum (mg/L)	Phosphate (mg/L)	Alkalinity (mg/L)
PT1	6.19±0.11	28.82±0.49	6.52±0.03	4.80±0.01	1.48±0.02	4.79±0.01	50.33±0.58
PT2	5.66±0.14	30.49±0.54	6.43±0.02	1.96±0.01	0.83±0.02	4.46±0.03	46.67±1.15
PT3	3.89±0.19	29.65±0.64	6.11±0.09	1.90±0.02	0.90±0.02	4.50±0.03	34.33±2.08
PT4	3.65±0.1	30.74±1.03	6.03±0.12	2.17±0.03	0.91±0.02	3.54±0.02	42.33±2.08
PT5	3.72±0.11	29.6±1.05	6.15±0.06	2.00±0.02	0.89±0.01	3.54±0.02	41.33±1.53
PT6	3.50±0.07	29.72±0.85	5.94±0.07	1.91±0.01	0.90±0.01	3.48±0.02	42.67±2.08
PT7	7.35±0.13	28.67±0.63	6.49±0.07	4.41±0.01	1.27±0.02	4.26±0.04	47.00±2.00
PT8	3.82±0.04	29.19±0.62	6.12±0.03	1.08±0.03	1.28±0.02	0.72±0.03	18.00±1.00
PT9	6.28±0.03	28.27±0.89	6.34±0.06	0.81±0.02	1.14±0.01	0.43±0.02	18.00±1.01
PT10	3.86±0.04	28.85±0.42	6.38±0.03	4.00±0.02	1.11±0.02	3.95±0.02	45.67±1.15
PT11	3.98±0.11	28.74±0.11	6.49±0.08	4.23±0.03	1.21±0.03	3.90± 0.03	43.33±0.58
PT12	4.21±0.1	28.99±0.19	6.38±0.02	4.46±0.05	1.26±0.04	3.81±0.04	46.00±1.00

A total of 21 fish species comprising 16 families (Loricariidae, Gobiidae, Megalopidae, Parastacidae, Channidae, Clariidae, Palaemonidae, Gecarcinucidae, Eleotridae, Cyprinidae, Pangasiidae, Anabantidae, Notopteridae, Cichlidae, Bagridae and Synbranchidae) were recorded during the study and its abundance was summarized in Table 3. Parastacidae is the dominant family in terms of species, representing 45.40%, followed by Palaemonidae (31.28%) and Gecarcinucidae (13-49%). The other families only constitute about 0.09-2.59% of the total species recorded in this study. Out of 21 fish species obtained at the 12 selected stations, 14 species were native, while seven (*Cherax quadricarinatus*, *Hypostomus plecostomus*, *Clarias gariepinus*, *Oreochromis niloticus*, *Pangasius* sp., *Barbonymus gonionotus* and *Amphilophus citrinellus*) were alien or non-native. Our findings compliment the fish landings data in 2016 and 2017 from Sg. Perai obtained from the Penang State Fisheries Office (Figure 4).

The dominant families recorded in this study is quite distinctive from other studies. Md Sah et al. (2012) recorded Cyprinidae as the dominant (56.0%) fish species family at the river feeders of Muda Reservoir. Meanwhile, Hashim et al. (2012) disclosed 57.1% of the total species caught from Sg. Enam and Sg. Telang, Temenggor Reservoir, belongs to the family Cyprinidae. Cyprinidae as a major fish family was also reported in an earlier study at Sg. Pelus Perak (Ikhwanuddin et al., 2016), Sg. Chantek, Besut, Terengganu (Ahmad et al., 2018), Kuala Tahan, Pahang (Farinordin et al., 2016), streams within Sungkai Wildlife Reserve, Perak (Ahmad et al., 2014) and nearby Ulu Paip Eco Forest, Kedah (Aqmal-Naser & Ahmad, 2021).

All these observations were in agreement with previous work by Chong et al. (2010) which imply that Cyprinidae is the dominant freshwater fish family in Malaysia. Cyprinidae might have dominated Sg. Perai years before and only recently being outnumbered by alien species such as those observed in this study. Our finding revealed that crustaceans are more dominant than fish in Sg. Perai. Nevertheless, the species checklist obtained in this study might not be as comprehensive due to the limitations in the fishing gear and sampling frequency carried out.

Cherax quadricarinatus recorded the highest number of catches and percentage, n=508 (45.4%), followed by giant freshwater prawn (*Macrobrachium rosenbergii*) (n=350, 31.3%) and rice crab (n=151, 13.5%). Our finding supports the claim that Sg. Perai is famous for the freshwater prawn. In another report, *M. rosenbergii* was the third-highest landing species from the middle zone of Sg. Muda river basin (Anon, 2019). Various literatures have described the importance of freshwater prawns as key organisms in tropical stream communities, as their foraging activities influence the ecology of benthic organisms such as algae and aquatic insects (Crowl & Covich, 1994). Degradation of organic litter by the prawns is vital as attainable nutrients for macroconsumers, primary producers and bacteria that contribute to the food-web structures (Covich et al. 1999). Hence, the presence of *M. rosenbergii* in Sg. Perai should be sustained as it could be of key importance to its health status. The low number of fish species observed in this study advocate that the fish species might not tolerate the environmental conditions and thus have a lower chance of surviving.

Fish composition by sampling stations is presented in Table 4. Station PT1 recorded the highest number of aquatic species present (14), followed by PT12 (11), PT7 (8) and PT8 (7) and PT3 (7). *M. rosenbergii* was found in most sampling stations (10 out of 12) i.e. PT1, PT2, PT3, PT4 in the upstream, PT7, PT8, PT9, PT10, PT11 and PT12 in the downstream and not in the middle part of Sg. Perai (PT5 and PT6). PT12 (n=243) recorded the highest count of *M. rosenbergii*, followed by PT11 (n=34). Presence of *Sayamia sexpunctata* was recorded in all sampling stations but outnumbered compared to *M. rosenbergii* and *C quadricarinatus*. *Pseudogobiopsis oligactis*, *Monopterus albus*, *Barbodes binotatus* and *Arius* sp. were the least species detected and only at one particular station.

Table 3. Fish composition recorded in Sg. Perai during the sampling period.

Local Name (English Name)	Scientific Name	Family	No of Individual (n)	Percentage (%)
Bandaraya (Armour catfish)	<i>Hypostomus plecostomus</i>	Loricariidae	29	2.59
Belungkor pasir (River goby)	<i>Pseudogobiopsis oligactis</i>	Gobiidae	1	0.09
Bulan (Indo-Pacific tarpon)	<i>Megalops cyprinoides</i>	Megalopidae	4	0.36
Lobster air tawar (Redclaw crayfish)	<i>Cherax quadricarinatus</i>	Parastacidae	508	45.40
Haruan (Striped snakehead)	<i>Channa striata</i>	Channidae	7	0.63
Keli Afrika (African catfish)	<i>Clarias gariepinus</i>	Clariidae	13	1.16
Keli bunga (Bighead catfish)	<i>Clarias macrocephalus</i>	Clariidae	3	0.27
Ketam padi (Freshwater crab)	<i>Sayamia sexpunctata</i>	Gecarcinucidae	151	13.49
Ketutu (Marble goby)	<i>Oxyeleotris marmorata</i>	Eleotridae	8	0.71
Lampam Jawa (Javanese barb)	<i>Barbonymus gonionotus</i>	Cyprinidae	9	0.80
Patin (Silver striped catfish)	<i>Pangasius</i> sp.	Pangasiidae	2	0.18
Puyu (Climbing perch)	<i>Anabas testudineus</i>	Anabantidae	4	0.36
Selat (Bronze featherback)	<i>Notopterus notopterus</i>	Notopteridae	10	0.89

Tebal sisik (Spotted barb)	<i>Barbodes binotatus</i>	Cyprinidae	1	0.09
Terbul (Bonylip barb)	<i>Osteochilus vittatus</i>	Cyprinidae	4	0.36
Tilapia hitam (Black tilapia)	<i>Oreochromis niloticus</i>	Cichlidae	10	0.89
Udang galah (Giant freshwater prawn)	<i>Macrobrachium rosenbergii</i>	Palaemonidae	350	31.28
Red cichlid (Midas cichlid)	<i>Amphilophus citrinellus</i>	Cichlidae	1	0.09
Enge enge	<i>Mystus singaringan</i>	Bagridae	2	0.18
Belut (Asian swamp eel)	<i>Monopterus albus</i>	Synbranchidae	1	0.09
Baung sungai (Yellow catfish)	<i>Hemibagrus capitulum</i>	Bagridae	1	0.09

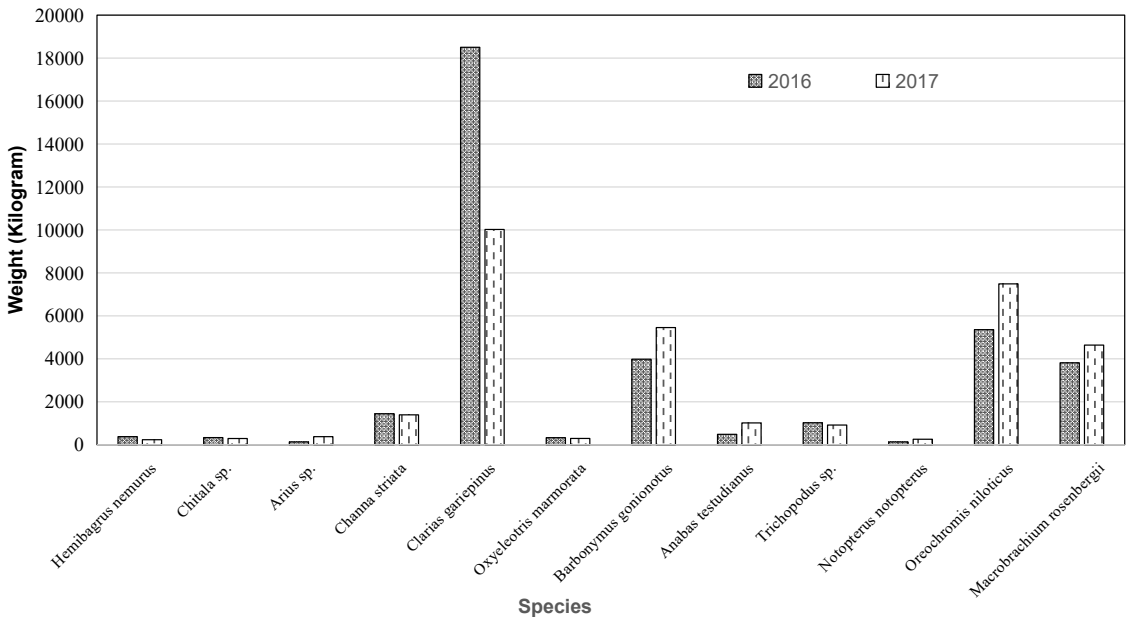


Figure 4. Sg. Perai fish landing data from 2016-2017 (Data from Penang State Fisheries Office).

Table 4. Fish composition recorded by sampling stations.

	PT1	PT2	PT3	PT4	PT5	PT6	PT7	PT8	PT9	PT10	PT11	PT12	Grand Total
<i>Amphilophus citrinellus</i>							1						1
<i>Anabas testudineus</i>								2			1	1	4
<i>Arius</i> sp.	1												1
<i>Barbodes binotatus</i>	1												1
<i>Barbonymus gonionotus</i>			3					1	4			1	9
<i>Channa striata</i>												7	7
<i>Cherax quadricarinatus</i>	118	131	112	22	53	72							508
<i>Clarias gariepinus</i>	2		2				1	2	1	3	2		13
<i>Clarias macrocephalus</i>	2	1											3
<i>Hemibagrus nemurus</i>			1										1
<i>Hypostomus plecostomus</i>	9	3	2		2	2	4	4				3	29
<i>Macrobrachium rosenbergii</i>	11	13	8	2			14	14	1	10	34	243	350
<i>Megalops cyprinoides</i>							4						4
<i>Monopterus albus</i>						1							1
<i>Mystus singaringan</i>	1					1							2
<i>Notopterus notopterus</i>	2	1				1	3					3	10
<i>Oreochromis niloticus</i>	2				3					1		4	10
<i>Osteochilus vittatus</i>	3											1	4
<i>Oxyeleotris marmorata</i>	2	1					3		1			1	8
<i>Pangasius</i> sp.	1							1					2
<i>Pseudogobiopsis oligactis</i>												1	1
<i>Sayamia sexpunctata</i>	34	20	20	11	16	23	6	5	2	4	8	2	151
Grand Total	189	170	148	35	74	100	36	29	9	18	45	267	1120

Presence of alien species

C. quadricarinatus is the most commonly encountered alien species in Sg. Perai. This species is a species native to river catchments in northern Australia and south-eastern Papua New Guinea (Lawrence & Jones, 2002). It is a non-burrowing and physically robust species that grows relatively quickly in a wide range of environments (Wingfield, 2002). The broad tolerance to environmental factors and attractive colours makes the species ideal for both aquaculture and the aquarium industry

and translocated to various locations within the Australia and Oceania region. This finding is quite disturbing as *C. quadricarinatus* is known to be highly invasive. Their dominance may not be comprehended because the local fishermen mainly used cast nets and splints in their operation and hence not commonly harvested. *C. quadricarinatus* was only detected in Sg. Jarak tributary (PT1, PT2, PT3 and PT4) and Sg. Perai main river (PT5 and PT6) which is upstream of Sg. Perai and not in other stations. The most frequently found sizes are between 11-20 g (n=103), 21-30 g (n=96) and 31-40 g (n=71) (Figure 5). Female *C. quadricarinatus* were commonly encountered (n=351) compared to males (n=146) with 11 (3.1%) of them being spawning females (Figure 6 and 7). Our finding records the first reporting of *C. quadricarinatus* in Sg. Perai, Penang. It adds to the other evidence on the proliferation of *C. quadricarinatus* in public waters of Malaysia since its introduction including in the southern (Johor) and west coasts of Peninsular Malaysia (Selangor) and Borneo Island (Sarawak) (Johan et al., 2012), Machap Dam, Sungai Benut (Johor), Ayer Keroh Lake (Melaka), Timun River (Melaka), Perdana Lake (Selangor) and Suai River (Sarawak) (Naqiuddin et al., 2016). Meanwhile, the first wild population of *C. quadricarinatus* on the East Coast of Peninsular Malaysia was recorded in Besut, Terengganu (Norshida et al., 2021). The existence of the crayfish has caught the attention of fishermen as they suffer losses due to the damage caused to their catch and fishing nets.

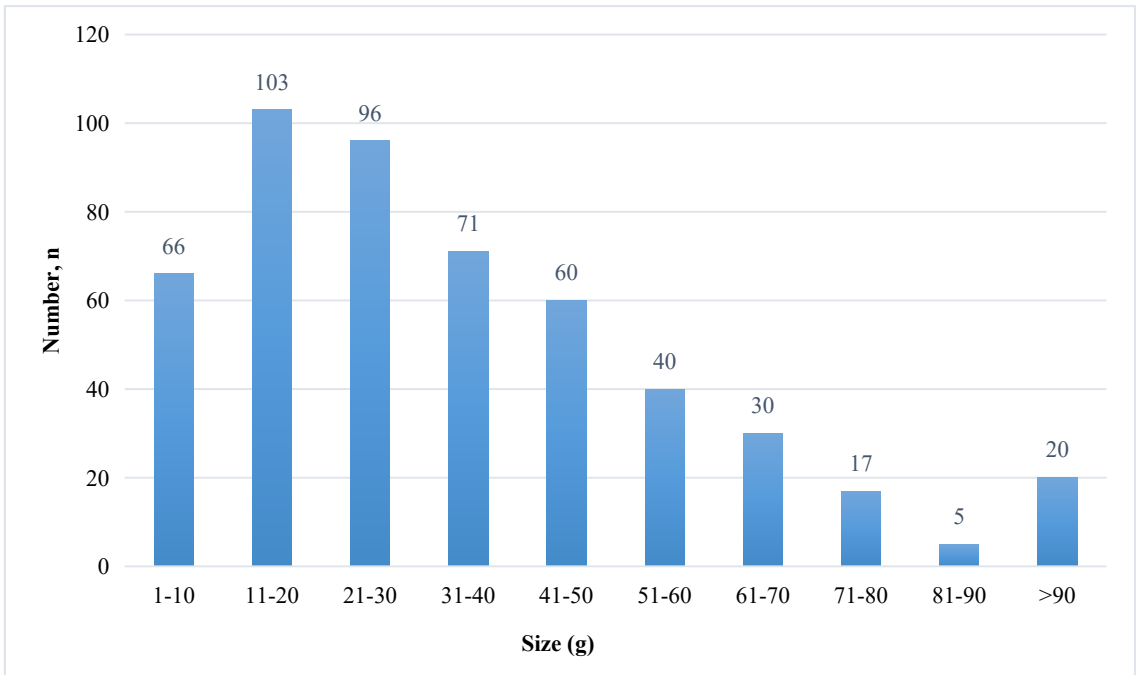


Figure 5. *Cherax quadricarinatus* size ranges observed in Sg. Perai.

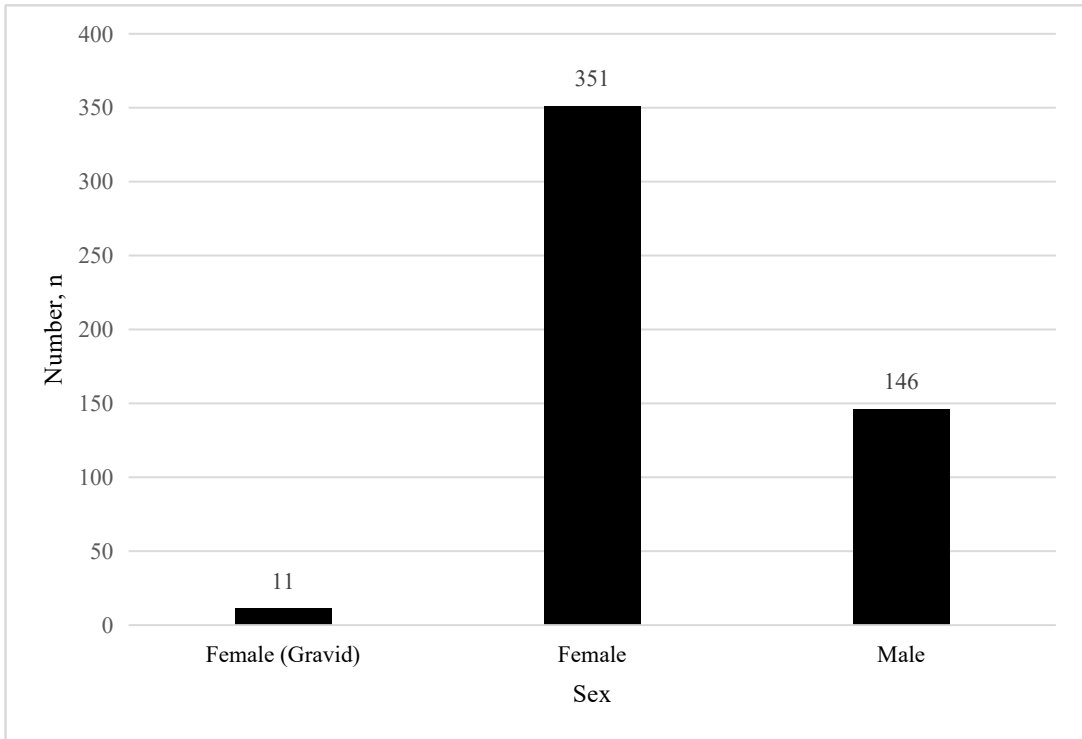


Figure 6. *Cherax quadricarinatus* males and females recorded at Sg. Perai.



Figure 7. *Cherax quadricarinatus* found in Sg. Perai.

C. gariepinus and *O. niloticus* were the other two alien species commonly encountered in this study. However, in Malaysia and many other countries, these two species are not listed as invasive because of their economic importance and their contribution to food security. Tilapia and catfish contributed significantly to aquaculture productivity in Malaysia with 31,790.24 tonnes (RM 1,632,593.41) and 39,815.33 (RM 3,074,849.02) production in 2022 (Department of Fisheries, 2023). The presence of catfish in Sg. Perai could have been a single cohort released simultaneously during religious rituals of releasing fish into the river to purify one's soul or to commemorate the birthday of a certain God.

The presence of the popular aquarium fish, the Armor catfish, *H. plecostomus*, in Sg. Perai suggested that this species was well established and self-maintained, as proposed by Khairul Adha (2012) and Khairul Adha et al. (2013).

The fifth common alien species detected (4 out of 12 sampling stations) is Javanese barb (*B. gonionotus*), *Pangasius* sp. and *A. citrinellus* were the least alien species detected. Javanese barb and *Pangasius* sp. have long been introduced in nearby Tasik Muda, Kedah (Lee et al., 2013). The presence of a hybrid fish, the midas cichlid (*A. citrinellus*), was recorded in this study but only in one station, PT7. Although there was no evidence of self-sustaining populations of this hybrid fish in Sg. Perai, their existence still poses the risk of food and space competition with native species as well as the spread of disease. Our finding was not unprecedented as the existence of the *A. citrinellus* and the associated hybrid in nearby Seberang Perai Tengah rice agroecosystem was previously reported (Aqmal-Naser & Ahmad, 2020).

Fish diversity of Sg. Perai

The Shannon Diversity Index (Shannon Wiener Index) is represented as H' in Sg. Perai is 1.47. The H' index is a method to measure the diversity of species in a community. A higher value of H' means a higher diversity of species in that community and a lower value of H' indicates a low diversity. If the H' value is equal to 0, this means that the community has only one species. These results suggest that species diversity in Sg. Perai is still high. In addition, the results of the Species Evenness Index (Pielou's Evenness Index) of Sg. Perai recorded a value of 0.47. Pielou's evenness is an index that measures diversity and species richness at once. Species richness is the number of different species in an area, while evenness is the number of individuals in each species in an area. Species Evenness Index values range from 0 (no evenness) to 1 (perfect evenness). The value of the Species Richness Index (Margalef's Richness Index) (DMg) was recorded at 6.89. DMg is a measurement of species diversity in an area or sample that is calculated based on the species that appear and the total number of individuals of the species that appear. Table 3 lists the Shannon-Wiener Diversity and Evenness Index of fish in several locations in the state of Penang and Perak for comparison purposes. The Diversity and Evenness Index in the present study was lower than other rivers. This is understood as other studies were carried out much earlier. The Diversity and Evenness Index in the rice field was low because the habitat of the rice field was not the same as rivers.

Table 3. The reported Shannon-Wiener Diversity and Evenness Index of fish in the north of the Peninsular Malaysia.

Location	ShannonWiener Index	Pielou's Evenness Index	Margalef's Richness Index	References
Sg. Perai, Penang	1.47	0.47	6.89	Present study
Sg. Telang, Perak	2.74	0.64	Not reported	Hashim et al. (2012)
Sg. Enam, Perak	1.69	0.49	Not reported	
Bersia Reservoir, Perak	2.030	0.732	Not reported	Hamid & Mansor (2013)
Temenggor Reservoir, Perak	1.921	0.653	Not reported	
Muda rice fields, Kedah	0.8764	0.8416	Not reported	Shah et al. (2010)

Future directions

Reducing the impact of *C. quadricarinatus* is crucial for protecting the biodiversity and ecosystems of Sg. Perai. To achieve this, several mitigation strategies could be carried out. Among the crucial ones are regular surveys to determine the latest update of the biodiversity. This could

be accomplished through engaging local communities and stakeholders in reporting sightings of *C. quadricarinatus* which may also impart real-time data for rapid response. Further studies are required in quantifying the impact of *C. quadricarinatus* on the ecosystem of Sg. Perai and also the financial impact or economic cost analysis of *C. quadricarinatus* on *M. rosenbergii* fisheries. In addition, a study on how climate change influences the spread of invasive aquatic species in Sg. Perai would help in acquiring a more nuanced understanding and enabling robust mitigation strategies.

In order to restrain further negative impacts of Sg. Perai, the fisheries resource manager could introduce natural predators such as *Anabas testudineus* (climbing perch), to manage the presence of *C. quadricarinatus* larvae, since they can reproduce exclusively in freshwater. Our preliminary study indicates that climbing perch is effective in reducing *C. quadricarinatus* larvae. Encouraging the fishermen to increase landings of redclaws in Sg. Perai can serve a dual purpose; not only does it help to remove them, but also provides an opportunity to generate additional income.

Conclusion

This study provides information and a preliminary overview of the composition and distribution of fish in Sg. Perai, where foreign species of redclaw crayfish are dominant, and the population shows a comfortable reproduction. A total of 21 fish species comprising 16 families were recorded during the study period, with Parastacidae as the dominant fish family. Sg. Perai was found to be dominated by the invasive species *C. quadricarinatus*. The nature of *C. quadricarinatus* which has high tolerance to changes in water quality, makes it able to breed easily. Its nature that defends the area/territory gives competition to the local prawn species, i.e. in terms of space and food. The results of the study suggest that although species diversity is still high in Sg. Perai, the distribution of species is less even. Further studies are necessary to assess the impact of the presence of this species on the ecosystem of Sg. Perai. It is feared that if this matter is not dealt with properly, it will affect the freshwater prawn resources in Sg. Perai in the future. Thus, continuous monitoring could be undertaken to ensure conservation of native species in Sg. Perai and species diversity is preserved and will not deteriorate further.

Acknowledgments

This survey was supported by the FRI development fund. The authors would like to thank Dr. Mohd. Fariduddin Othman for his support in the survey implementation and Mr. Mustafa Asmuni for his expertise that greatly assisted this short study.

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Determining the optimal cooling rate for effective waterless transport of anesthetized tiger prawns (*Penaeus monodon*)

CHE ZULKIFLI CHE ISMAIL^{1*}, AIMIE RIFHAN HASHIM², NOR SHAFIDA MOHAMAD TARMIZI¹

¹ Fisheries Research Institute Pulau Sayak, 08500 Kota Kuala Muda, Kedah

² Faculty of Applied Sciences, Universiti Teknologi MARA Arau Branches, 02000, Arau, Perlis, Malaysia

*Corresponding author: che.zulkifli@dof.gov.my

Abstract: Waterless transportation of crustaceans is a method used to reduce the cost of transporting live animals, especially via airfreight. In this method, tiger prawns were packed in containers without water and rely on the moisture within the packaging for respiration. Cold temperatures act as an anaesthetic agent, reducing the prawns' metabolic rate and oxygen consumption. The cooling method for anesthetizing prawns is critical to ensuring their survival during waterless transport, as each species requires different cooling times. Tiger prawns (*Penaeus monodon*) are a significant export commodity for Malaysia. This study was conducted to determine the optimal cooling time for anesthetizing tiger prawns. The survival rate of tiger prawns was assessed after reintroducing them to seawater following waterless transport. The study found that the best cooling time, from 26°C to 15°C, was two hours, with a survival rate of 87%. The survival rate with a one-hour cooling time was 62.7%. There was no significant difference ($P>0.05$) in the survival rate of prawns between the two-hour cooling and the control group (transport with water as the medium).

Keywords: cooling protocol, cold anesthesia, *Penaeus monodon*, tiger prawns, waterless transportation

Abstrak: Pengangkutan krustasea tanpa air adalah kaedah yang digunakan untuk mengurangkan kos pengangkutan, terutamanya melalui pengangkutan udara. Dalam kaedah ini, udang harimau dibungkus dalam bekas tanpa air dan bergantung pada kelembapan dalam bungkusan untuk pernafasan. Suhu sejuk bertindak sebagai agen anestetik, mengurangkan kadar metabolisme dan penggunaan oksigen oleh udang. Kaedah penyejukan untuk membius udang adalah penting untuk memastikan kemandirian semasa pengangkutan tanpa air, kerana setiap spesies memerlukan masa penyejukan yang berbeza. Udang harimau (*Penaeus monodon*) merupakan komoditi eksport yang penting bagi Malaysia. Kajian ini dijalankan untuk menentukan masa penyejukan optimum untuk membius udang harimau. Kadar kemandirian udang harimau dinilai selepas udang dimasukkan semula ke dalam air laut selepas pengangkutan tanpa air. Hasil kajian mendapati bahawa masa penyejukan terbaik, dari 26°C hingga 15°C, adalah dua jam, dengan kadar hidup sebanyak 87%. Kadar hidup dengan masa penyejukan satu jam ialah 62.7%. Tidak terdapat perbezaan yang signifikan ($P>0.05$) pada kadar hidup udang antara penyejukan dua jam dan kumpulan kawalan (pengangkutan dengan air sebagai medium).

Introduction

In Malaysia, the tiger prawn (*Penaeus monodon*) is one of the most important marine shrimp species, second only to the Pacific white shrimp in terms of production. Most tiger prawn production is exported, primarily marketed in frozen form and shipped in refrigerated containers. Live transportation has emerged as a method to ensure product freshness and increase market value. However, it cannot

be transported in large volumes. The conventional practice for transporting live shrimp involves packing them in plastic bags filled with water and oxygen, then sealing them with rubber bands. The main contributing factor to the high cost of live shrimp transportation, especially in air freight, is the weight of the water. Reducing this weight can significantly lower transportation costs. Thus, waterless transport offers a cost-effective alternative by eliminating the need for water.

Waterless transportation is an increasingly popular method for transporting live crustaceans. Traditional transportation methods often cause stress in aquatic animals due to fluctuations in temperature and vibrations, which can negatively affect water quality and subsequently influence the animals' blood biochemistry, gill morphology, oxidative stress levels, and flesh quality (Fang et al., 2023). Waterless transport confines animals in containers without water, where they rely on moisture in the packaging and cold ambient temperatures to reduce oxygen consumption.

The cold temperatures serve as an anaesthetic agent, lowering the animal's metabolic rate and oxygen needs during transit. The cooling process is vital in waterless transport, as each species requires a specific cooling rate for effective anesthetization and to minimize stress and mortality. For example, the ideal anesthetization temperature for the red-spotted grouper (*Epinephelus akaara*) is between 20°C and 25°C, as these temperatures help reduce stress responses (Cho et al., 2015). Channel catfish, when gradually cooled by 2°C per hour, maintain physiological balance better than with rapid cooling (Bai et al., 2020). Warm-water shrimp, such as the Pacific white shrimp (*Litopenaeus vannamei*) and freshwater prawns (*Macrobrachium rosenbergii*), face challenges in waterless transport due to their sensitivity to low temperatures (Taylor & Kuhn, 2017). This study aims to determine the optimal cooling rate for cold anesthetization of tiger prawns in a waterless transport protocol.

Materials and Methods

Healthy live prawns were carefully selected and weighed to ensure 1 kilogram (40 individuals) per group (3 treatments; 3 replicates; 9 kg total). Each treatment used 25 L of filtered seawater, filled into a 30 L Styrofoam box, and prepared with vigorous aeration for chilling treatments. Initial parameters such as temperature, salinity, and dissolved oxygen (DO) were carefully measured and recorded. The experiment involved three different chilling methods, which are fast-cooling, intermediate-cooling, and slow-cooling, with the optimum temperature for anesthetization being determined as 15°C based on the initial results. For each cooling treatment, three replicates were performed using 1 kg of marketable-sized tiger prawns (*P. monodon*). The effectiveness or suitability of each cooling treatment for cold anesthetization was evaluated by measuring the percentage of surviving prawns at the end of a six-hour waterless transport simulation.

Fast cooling treatment

Three styrofoam boxes, each filled with 25 L of seawater, were prepared and the initial temperature was measured. The seawater temperature was reduced to 15°C before the prawns were introduced. Once the temperature stabilized at 15°C, the tiger prawns were added to the chilled seawater. The temperature was carefully monitored to ensure it did not drop below the target 15°C. One kilogram of tiger prawn (*P. monodon*) was introduced to the chilled seawater, and their physical activity was monitored until they were fully anesthetized. Once anesthetized, the tiger prawns were removed from the water and placed onto a thin layer of chilled, shaved wood inside a modified plastic container. Care was taken to avoid overcrowding (each container holding 0.5 kg of tiger prawn in 3 layers). Two containers (a total of 1 kg of tiger prawn) were then placed in double-layered plastic packaging filled with pure oxygen and sealed with a rubber band. The packaged tiger prawns were placed in the Styrofoam box along with four packs of ice, and the box was sealed with cotton tape. A

mock waterless transport simulation was conducted for 6 hours, after which the survival rate of the tiger prawn was recorded 15 minutes after placing them in a revitalization tank.

Intermediate cooling treatment (1 hour)

Three styrofoam boxes were again filled with 25 L of filtered seawater and aerated. One kilogram (40 individual) of tiger prawn was added to each box, and the initial temperature and dissolved oxygen levels were recorded. Over the course of 1 hour, the temperature was reduced, with measurements taken every 5 minutes. Ice packs (1 kg each) were added every 10 minutes until the seawater temperature reached 15°C. The tiger prawn's physical activity was observed, and they were removed from the water once fully anesthetized. The anesthetized tiger prawns were then placed onto chilled, shaved wood in a modified plastic container, ensuring no overcrowding (0.5 kg per container, in 3 layers). As with the fast-cooling treatment, the tiger prawns were packaged in double-layered plastic with pure oxygen and placed in a styrofoam box with four ice packs. After a 6-hour mock waterless transport simulation, the tiger prawns were placed into a revitalization tank, and survival rates were recorded 15 minutes later.

Slow cooling treatment (2 hours)

In the slow cooling treatment, the same method was followed, with 25 L of seawater and aeration in each styrofoam box. One kilogram (40 individual) of tiger prawn was added to each box, and initial measurements were recorded. Over 2 hours, the water temperature was gradually reduced, with measurements taken at 10-minute intervals. Ice packs were added every 20 minutes until the target temperature of 15°C was reached. The tiger prawn's physical activity was monitored, and once fully anesthetized, they were removed from the water and placed onto the chilled, shaved wood in the modified plastic containers, ensuring no overcrowding. The tiger prawns were then packaged, placed in styrofoam boxes with four ice packs, and subjected to a 6-hour waterless transport simulation. Afterward, survival rates were recorded 15 minutes after they were placed into the revitalization tank. Survival rates based on the tiger prawn that survived after the 6-hour mock transport were documented.

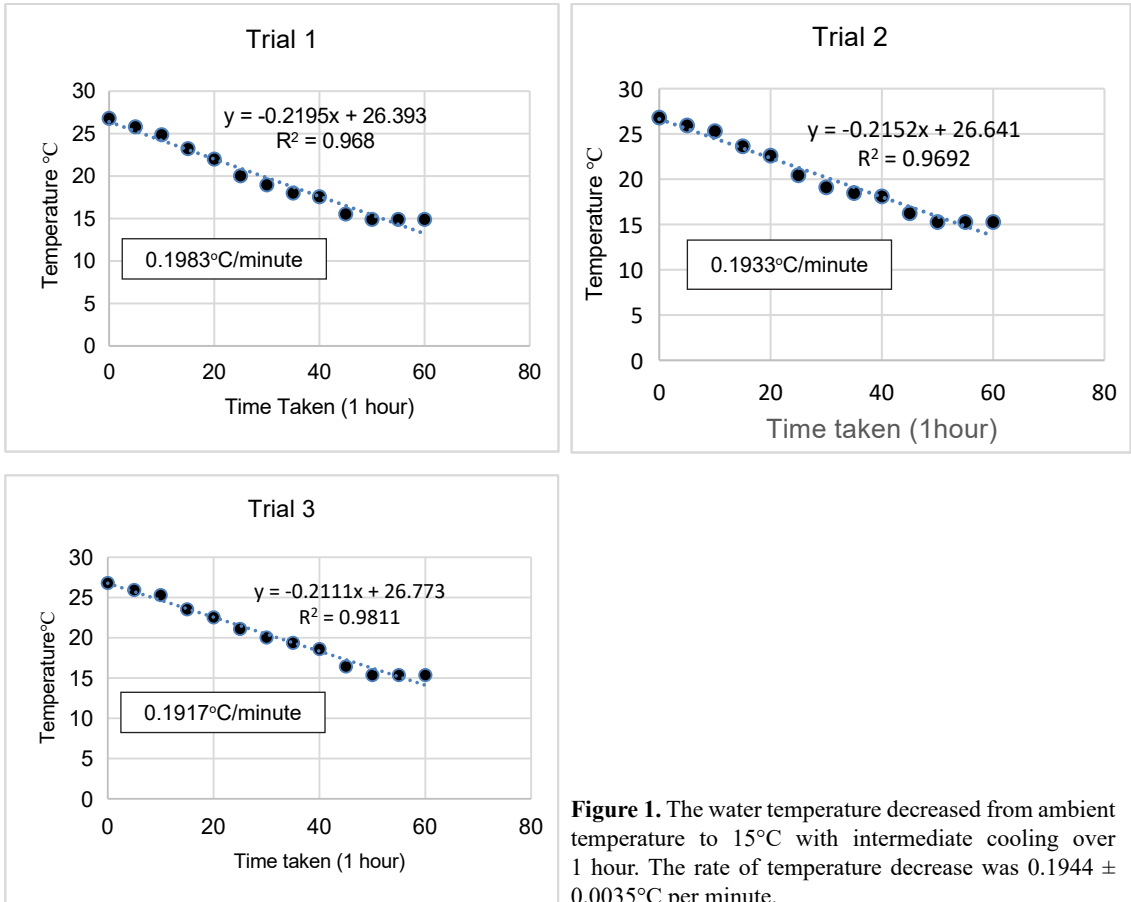
Results

Fast cooling

The survival rates across the three replicates were 4.25%, 2.50% and 19.05%, with an average survival rate of $8.60 \pm 0.09\%$ for the fast-cooling treatment.

Intermediate cooling (1 hour)

Figure 1 shows the results of three trials on the intermediate cooling of water containing tiger prawns. The temperature decreases from ambient water temperature to 15°C within one hour was $0.1944 \pm 0.0035^\circ\text{C}$ per minute. The survival rates for the three replicates were 72.1%, 65.9% and 69.1%, resulting in an average survival rate of $69.03 \pm 3.1\%$.



Slow treatment (2 hours)

Figure 2 shows the result after six hours of waterless transport simulation, the survival rates for the three replicates were 84.62%, 90.00%, and 86.49%, resulting in an average survival rate of 87.03% ± 0.027. This treatment yielded the highest survival rates compared to both the intermediate and fast-cooling treatments.

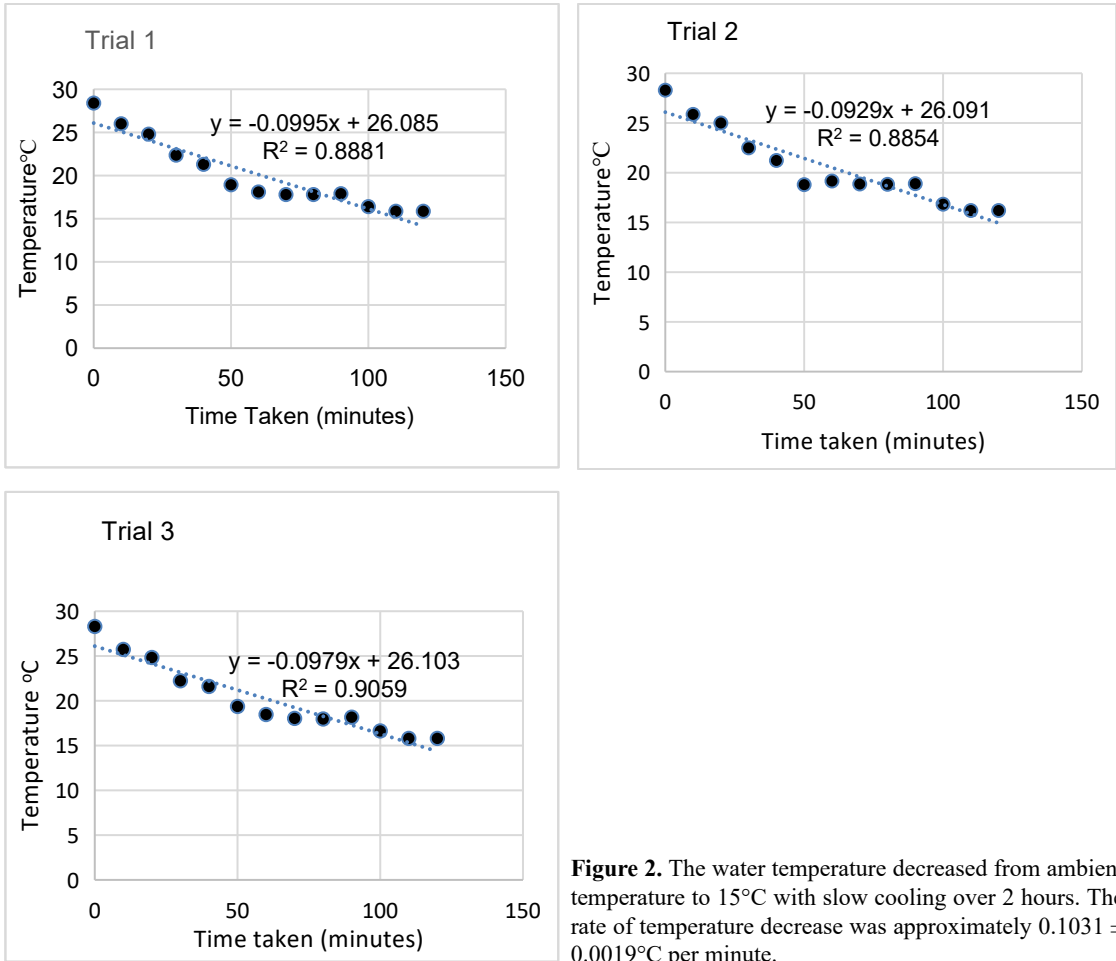


Figure 2. The water temperature decreased from ambient temperature to 15°C with slow cooling over 2 hours. The rate of temperature decrease was approximately $0.1031 \pm 0.0019^\circ\text{C}$ per minute.

Figure 3 shows the result of Experiment 1. Slow cooling is the best cooling method based on the survival rate of tiger prawns during waterless transportation. There is no significant difference ($P > 0.05$) between slow cooling and control, which is normal transportation for live prawns, which uses water. There is a significant difference ($P < 0.05$) in the survival rate of the prawns between slow cooling and intermediate cooling, and between slow cooling and fast cooling. Fast cooling results in the worst outcome, which is an exceptionally low survival rate.

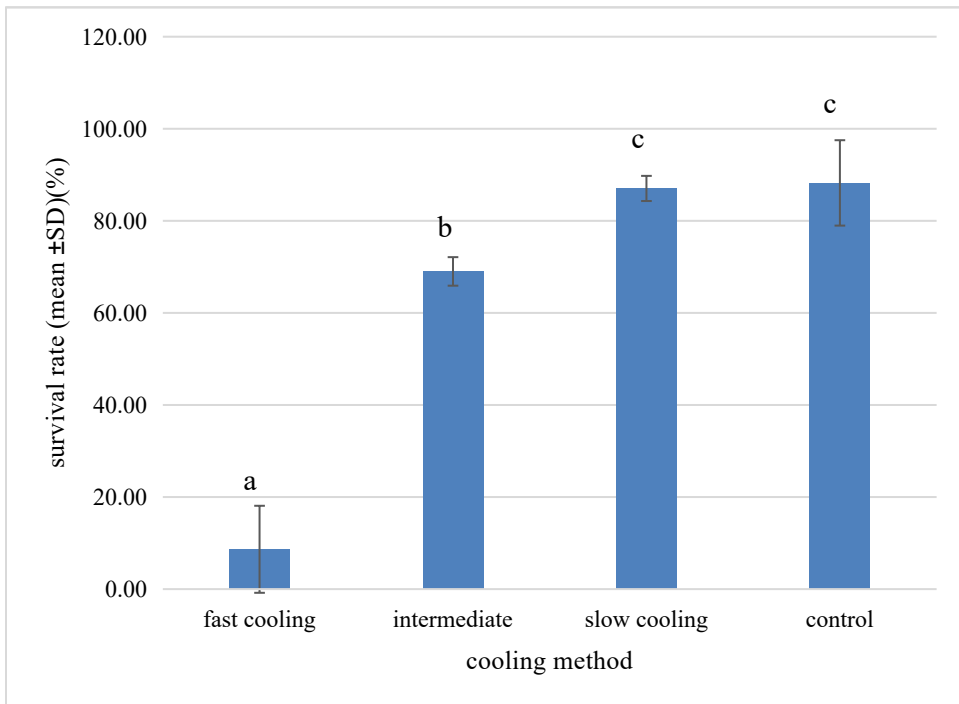


Figure 3. The survival rate of tiger prawns after waterless transport using fast, intermediate, and slow chilling methods. Same letters indicate values with non-significant differences ($P > 0.05$).

Discussions

The results from the chilling experiment show that the temperature at which the tiger prawns were anesthetized was 15°C. This finding is consistent with a study conducted by Salin (1997) on the same species, which reported an anesthetization temperature of 14°C. At this temperature, the prawns became inactive, and the packing process was carried out. Several factors likely influenced the survival rates during the chilling process. Primarily, the temperature of the cooling water and the size of the prawns played significant roles. Larger prawns exhibited greater tolerance to rapid temperature changes compared to smaller ones. While waiting for the larger prawns to be anesthetized, the smaller prawns may have already experienced extreme hypothermia, causing their cells to undergo osmotic shock, which ultimately led to death during the mock transport simulation. In fish, the success of waterless transportation relies on cold-induced dormancy (or hibernation). During this process, fish adjust to low temperatures by modifying the activity levels of metabolic enzymes and altering the phospholipid composition of their cellular membranes (Fan, 2019).

The underlying biochemical processes responsible for prawns' mortality during waterless live transport can be explained by morphological analysis and immune system assessments. Prawns exposed to rapid cooling and waterless transport are subject to oxidative stress, prompting them to activate antioxidative and immune defence mechanisms to recover (Xu et al., 2021). The size of the tiger prawn also affects their physiological response to cooling anaesthetics. Smaller tiger prawns display distinct growth responses compared to larger ones, which can influence their overall resilience to stressors like temperature changes (Ravaux et al., 2016). Additionally, the thickness of the chilled shaved wood layer should not be too great, as it can act as a barrier, limiting oxygen intake and causing the prawns to suffocate in the container. Proper handling during preparation also plays a crucial role in

the survival of the prawns. Prawns may experience injury or stress during the weighing and selection processes before anesthetization and packaging. Moreover, overcrowding or high prawn density in the packaging can lead to suffocation and death during transport.

Conclusion

The most effective cooling rate for anesthetizing tiger prawns in waterless transport is slow cooling over two hours. This method yields the highest survival rate (87.03%) compared to faster cooling rates.

Acknowledgement

We would like to thank the Division of Fisheries Security, Department of Fisheries Malaysia, for funding this study. Special thanks to the Director of the Fisheries Research Institute, Pulau Sayak, Mr. Mohammed Suhaimee Abd Manaf, for encouraging the publication of this article. We also express gratitude to all staff involved in the study.

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Mitochondrial DNA barcoding for identifying commercially important marine shrimp species in the waters of Kedah state, Malaysia

TEOH PIK NENG, CHE ZULKIFLI CHE ISMAIL, NOR AIDA SUZANA ABDUL RAHMAN,
SAADIAH IBRAHIM*

Fisheries Research Institute (FRI) Pulau Sayak, 08500 Kota Kuala Muda, Kedah, Malaysia

**Corresponding author: saadiah@dof.gov.my*

Abstract: Species identification has traditionally relied on taxonomic keys and the expertise of trained taxonomists. However, mitochondrial DNA barcoding, particularly targeting the *cytochrome c oxidase subunit I* (COI) gene, offers a precise and efficient alternative. In this study, tissue samples from five local marine shrimp species - green tiger shrimp (udang bunga/udang harimau hijau), jinga shrimp (udang kulit keras), banana shrimp (udang kaki merah), rainbow shrimp (udang belang) and yellow shrimp (udang cendana) were analysed using DNA barcoding. The mitochondrial COI gene sequences were obtained and processed with NGSspeciesID v3.0, then compared against the MIDORI2 database via BLASTn with an e-value threshold of 1e-100. The results confirmed the species identities as *Penaeus semisulcatus*, *Metapenaeus affinis*, *Penaeus merguensis*, *Parapenaeopsis sculptilis* and *Metapenaeus brevicornis*. This approach proves to be a simple, accurate and indispensable tool, particularly in the absence of trained taxonomists, ensuring reliable identification for research, particularly for broodstock development programs.

Keywords: Species identification, DNA barcoding, marine shrimp, taxonomy, MIDORI2 database

Abstrak: Pengenalpastian spesies secara tradisinya bergantung kepada maklumat taksonomi dan kepakaran ahli taksonomi terlatih. Walau bagaimanapun, kod bar DNA mitokondria dengan gen cytochrome c oxidase subunit I (COI) menawarkan alternatif yang tepat dan cekap. Dalam kajian ini, sampel tisu daripada lima spesies udang laut tempatan -udang bunga/ udang harimau hijau (green tiger shrimp), udang kulit keras (jinga shrimp), udang kaki merah (banana shrimp), udang belang (rainbow shrimp) dan udang cendana (yellow shrimp) dianalisis menggunakan kod bar DNA mitokondria. Jujukan gen COI mitokondria diperolehi dan diproses dengan NGSspeciesID v3.0, kemudian dibandingkan dengan pangkalan data MIDORI2 melalui BLASTn dengan ambang e-nilai 1e-100. Keputusan mengesahkan identiti spesies sebagai *Penaeus semisulcatus*, *Metapenaeus affinis*, *Penaeus merguensis*, *Parapenaeopsis sculptilis* dan *Metapenaeus brevicornis*. Pendekatan ini terbukti sebagai kaedah yang mudah, tepat dan amat diperlukan, terutamanya jika tiada ahli taksonomi terlatih, untuk memastikan pengenalpastian yang tepat untuk penyelidikan terutamanya bagi program pembangunan baka.

Introduction

Several commercially important shrimp species are landed by coastal fishermen from the water of Pulau Sayak and Kuala Kedah, Peninsular Malaysia. These species include the green tiger shrimp (*Penaeus semisulcatus*), jinga shrimp (*Metapenaeus affinis*), banana shrimp (*Penaeus merguensis*), rainbow shrimp (*Parapenaeopsis sculptilis*) and yellow shrimp (*Metapenaeus brevicornis*), which are locally known as udang bunga/udang harimau hijau, udang kulit keras, udang kaki merah, udang belang and udang cendana, respectively. For the purpose of this study, the term “shrimp” encompasses

both marine and brackish species of commercial interest. These shrimps show significant potential for development as aquaculture species, alongside commercially farmed Pacific white shrimp and tiger shrimp, which are typically imported strains from broodstock multiplication centres.

Despite the abundance of local shrimp, their genetic identities remain undetermined. Accurate taxonomic identification is essential to unlock their aquaculture potential. To achieve this, the mitochondrial DNA *cytochrome c oxidase subunit I* (COX1) gene is sequenced and compared against the MIDORI2 database. This approach has been widely employed for the taxonomic identification of diverse species, including red algae, nematodes, protozoa, crayfish and orchids (Patil et al., 2023; Traversa et al., 2017; Rodrigues et al., 2017; Tan et al., 2020; Wang et al., 2023). The objective of this study is to accurately identify local shrimp species of commercial interest using the COX1 gene sequencing. This identification is critical for advancing research on breeding, larval rearing and broodstock development programs.

Materials and Methods

Sample collection

Jinga shrimp, rainbow shrimp, yellow shrimp and banana shrimp were caught using three-layer nets by coastal fishermen in the waters of Pulau Sayak and Kuala Kedah (Figure 1). Meanwhile, green tiger shrimp were collected from deeper waters off the coast of Kuala Kedah. Figure 2 shows photographs of the selected species used in this study. The shrimp samples were sent to the laboratory of the Fisheries Research Institute (FRI) Pulau Sayak. Using aseptic techniques, 0.5 g of fresh tissue from each shrimp was collected, preserved in 1.5 mL of absolute ethanol and transported to a private laboratory, Patriot Biotech Sdn Bhd, for nucleic acid extraction, sequencing, taxonomic classification and phylogenetic analysis.

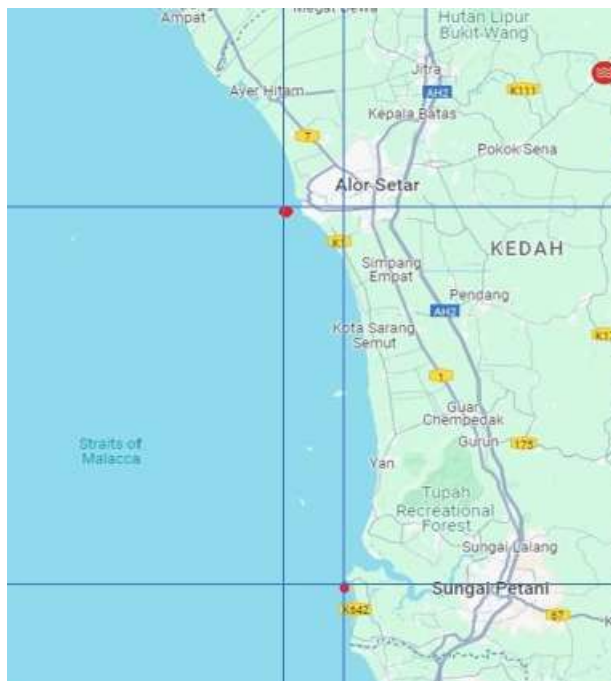


Figure 1. The red mark shows the site where the shrimp samples were collected. Kuala Kedah (6.098906, 100.264861), Pulau Sayak (5.644000, 100.321165).



Figure 2. The selected shrimp species used in this study.

DNA extraction

Approximately 50-150 mg of shrimp muscle tissue was finely crushed and had their gDNA extracted using the conventional SDS salting-out method. Briefly, the crushed tissues were placed into 500 μ L of lysis buffer (1% SDS, 150 mM NaCl, 25 mM Tris-HCl, pH 8, 10 mM EDTA) containing 5 μ L Proteinase K and incubated at 55°C for 1 hour. The lysate was then mixed with 1/3 volume of saturated NaCl and incubated at 4°C for 10 minutes to precipitate protein. After centrifugation at 10,000 x g for 5 minutes, the supernatant were mixed with 0.6x volume of isopropanol to precipitate gDNA. The gDNA pellet was washed once with 70% ethanol to remove salt, dried for 10 minutes and resuspended with 50 μ L of TE buffer (10 mM Tris-HCL pH 8, 1 mM EDTA). The extracted gDNA was also assessed on the gel for integrity on a 1% agarose gel.

COX1 gene amplification

The COX1 gene was amplified using Folmer degenerate primers of TNTCAACNAAYCAYAARGAYATYGG (Forward primer) and TANACYTCNGGRTGNCCRAARAAYCA (Reverse primer) were used (Geller et al., 2013). PCR reaction was conducted with 1 μ L of gDNA, 25 μ L of 1x Taq PCR Super Master Mix (Tinnzyme, China) and primers at a final concentration of 0.25 μ M each. The thermocycling conditions were initial denaturation at 95°C for 3 minutes. Then, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 45°C for 30 seconds and extension at 72°C for 30 seconds. The final extension was at 72°C for 5 minutes. The PCR products were visualized on a 1% agarose gel to confirm successful amplification.

COX1 gene sequencing

The COX1 gene amplicon was purified with 0.6x vol of Ampure Bead (Beckman Coulter, USA) and barcoded individually using the PCR Barcoding Expansion 1-12 (Oxford Nanopore, UK) according to the manufacturer's instructions, followed by library preparation using the LSK114 Q20+ kit (Oxford Nanopore, UK). The library containing the barcoded COX1 gene amplicon, ligated to the motor protein, was sequenced on a Flongle flowcell for 24 hours to generate the raw pod5 signal for subsequent processing. Basecalling (conversion of raw pod5 signal to fastq file) and demultiplexing of the data used dorado v0.7.3 (<https://github.com/nanoporetech/dorado>) and the super accuracy mode.

Sequence processing and analysis

Primer sequences were trimmed from demultiplexed reads using Cutadapt v4.6 with a maximum error rate of 0.15. The trimmed reads were analysed using NGSpecies ID v3.0 (Sahlin et al., 2021) to generate COX1 sequence consensus for each sample. Full-length consensus sequences were searched against the MIDORI2 database using BLASTn with an e-value threshold of 1e-100. For each amplicon, the top 10 hits were identified. The sequences were aligned using MAFFT (with --adjust direction and --maxiterate 1000 --localpair settings) and a maximum likelihood tree was constructed using FastTree (with --gtr and --nt settings) (Kato et al. 2002; Price et al 2010).

Results and Discussion

The results for COX1 sequences for the shrimp samples are shown in Table 1 and the tree diagram showing relationships among various shrimp samples in Figure 3.

Table 1. The COX1 sequences for the shrimp samples.

<p>>Rainbow shrimp or udang belang</p> <p>TACACTATATTTTATCTTTGGAGCTTGAGCAGGTATAGTTGGAAGCTGCATTAAGCTTAATTATC-CGAGCCGAATTAGGTCAACCTGGTAATCTTATTGGAGATGACCAAATTTATAATGTTGTCGTTAC-CGCCCACGCATTTGTTATAATTTCTTTATAGTTATACTATAATAATTGGAGGATTCGGAAATT-GATTAGTTCCCTCTTATATTAGGAGCCCCGATATAGCCTTCCCCCGAATAAATAATATAAGCTTCT-GATTACTTCCCCCATCATTAACTCTACTCCTATTAAGAGGTATAGTTGAAAGTGGAGTTGG-TACAGGCTGAACCGTTTATCCTCCTTAGCAAGAGGAATTGCCCATGCAGGAGCCTCAG-TAGATATAGGAATCTTTTCATTGCATTTAGCCGGAGTCTCATCCATTTTAGGAGCCGTAAACTTCATA-ACTACAGTAATCAACATACGATCATCTGGAATAACAATAGATCGTATACTTTTATTCGTCTGAT-CAGTTTTTATCACCGCCCTATTACTACTCCTCTCTTCCAGTTCCTAGCCGGAGCTATTACTATACTTCT-TACTGACCGAAATCTAAATACATCTTCTTTGACCCTGCCGGTGGTGGTGACCCAATCCTCTACCAA-CATTTATTC</p>
<p>>Yellow shrimp or udang cendana</p> <p>AACTTTATATTTTATTTTCGGAGCTTGAGCTGGAATAGTAGGTACTGCTTTAAGTTTAATTATCCGAG-CCGAAGCTGGTCAACCAGGTAGACTTATTGGAGACGATCAAATTTATAATGTTGTAGTTACCGC-CCACGCTTTTGTATAATTTCTTTATAGTTATAACCAATTATGATTGGTGGATTTGGTAATTGACTTGTC-CCTCTTATACTCGGAGCACCCGATATAGCATTCCCACGAATAACAATATGAGTTTTTGACTACTTC-CACCATCCTTAACACTCCTTCTTTCTAGTGGAATAGTAGAAAGAGGTGTAGGAACAGGATGAACG-GTTTTATCCTCCCTTAGCAGCTGGAATTGCCACGCAGGAGCTTCAGTTGATATAGGAATTTTTTCTC-TACATCTTGCTGGAGTTTCATCTATTTTAGGAGCAGTTAATTTTATAACAACAGTCATTAACAT-ACGCCCTGCTGGAATAACTATAGACCGTATACCACTTTTTGTATGGGCCGTATTTATTACAGCCT-TACTTCTTTTATTATCACTACCAGTTTTAGCTGGGGCTATCACTATGCTTTTAACAGACCGAAACCTTAA-TACATCCTTTTTCGATCCCGCTGGAGGAGGAGATCCAATTCTATACCAGCATTTATTT</p>
<p>>Green tiger shrimp or udang harimau hijau</p> <p>AACATTATATTTTCAATTTTCGGTGCTTGAGCTGGAATAGTAGGTACAGCTCTTAGACTTATTATTC-GTGCTGAATTAGGTCAACCTGGTAGACTTATTGGAGATGATCAAATTTATAATGTTGGTTGTAA-CAGCTCACGCTTTTGTATAATTTCTTCATAGTTATACCTATCATGATTGGAGGATTTGGTAACT-GACTAGTTCCCTCTAATATTAGGAGCTCCAGATATAGCTTCCCTCGTATAAATAATATAAGCTTCTG-GCTTTTACCTCCTTACTAACCTTACTTTTATCTAGAGGTATAGTAGAAAGAGGAGTAGGAACAGGTT-GAACAGTATAACCTCCTTTATCTGCCAGAATTGCTCACGCAGGTGCTTCAGTAGACTTAGGGATCTTCT-CACTTCATCTAGCAGGTGTATCATCTATTTTAGGAGCCGTAAATTTATAACAACCGTTATTAATATAC-GATCTACTGGAATAACTATAGACCGAATACCTCTGTTCTGTTGAGCAGTATTATTACTGCCCTGCTTC-TACTTCTATCTTTACCAGTGCTAGCAGGAGCTATTACAATGCTTCTAACAGACCGAAATCTAAATA-CATCCTTCTTCGACCCTGCCGGTGGAGGAGACCCTGTACTATATCAACACTTATTT</p>

>Banana shrimp or udang kaki merah

AACCTTATACTTTATCTTCGGAGCCTGAGCTGGAATAGTAGGGACTGCCCTTAGACTTATTATTC-
GTGCTGAATTGGGTCAACCGGAAGCCTCATTGGAGATGATCAAATTTATAATGTGGTTGTTA-
CAGCCCACGCTTTCGTTATAATTTCTTCATGGTAATACCTATTATAATGGAGGATTTGGAAAT-
GACTAGTCCCTTTAATATTAGGTGCTCCCGATATAGCTTTCCCCGAATGAATAATATGAGTTTTT-
GACTTTTACCTCCTTCACTAACTCTACTTCTTTCTAGAGGTATAGTTGAAAGAGGAGTAGGAACAGGAT-
GAACTGTTTACCCTCCTCTATCTGCCAGCATCGCCATGCAGGAGCATCTGTAGACCTAGGAATCTTCT-
CATTACATTTAGCAGGGGTTTCTTCAATTCAGGTGCTGTAAATTTATAACAACCTGTTATCAATATAC-
GATCAACAGGAATAACTATAGACCGAATACCTCTTTTCGTCTGAGCGGTATTTATTACAGCCTTAC-
TACTTTTACTATCATTACCAGTTTTAGCGGGAGCTATTACAATGCTTCTAACGGACCGGAACCTAAA-
TACTTCATTCTTCGATCCTGCAGGAGGGGGAGATCCTGTTTTATATCAACACTTATTT

>Jinga shrimp or udang kulit keras

AACCTTATATTTTATTTTCGGGGCTTGAGCCGGAATAGTTGGTACTGCTCTAAGTTTAATTATTCGTGC-
CGAACTAGGCCAACCAGGTAGTTTAAATTGGAGATGACCAAATTTATAATGTTGTAGTAACTGCCCAT-
GCCTTTGTTATAATTTCTTCATAGTTTATACCAATTATAATTTGGTGGATTTGGTAATTGACTCGTAC-
CCCTTATATTAGGAGCACCTGATATAGCATTCCACGTATAAACAATATAAGATTCTGACTTTTACCTC-
CATCTTTACACTACTCCTATCAAGAGGAATGGTAGAAAGAGGAGTCGGTACAGGATGAACCTGTG-
TATCCCCGCTAGCAGCAGGAATGGCCACGCTGGAGCCTCAGTTGACATAGGAATTTTTCAC-
TGCACCTCGCCGGGTCTCATCAATTCTAGGTGCAGTTAATTTTATAACAACCTGTTATTAACAT-
ACGCCCTGCAGGAATAACTATAGACCGTATACCACTTTTCGTATGAGCAGTATTTATTACAGC-
CCTACTCCTTCTTTTATCTCTACCAGTCTTAGCTGGAGCAATTACTATACTTCTAACTGACCGAAATCT-
TAACACCTCTTTCTTTGATCCAGCAGGAGGTGGAGACCCAATTCTATACCAACACTTATTT

>Jinga shrimp or udang kulit keras2

AACCTTATATTTTATTTTCGGGGCTTGAGCCGGAATAGTTGGTACTGCTCTAAGTTTAATTATTCGTGC-
CGAACTAGGCCAACCAGGTAGTTTAAATTGGAGATGACCAAATTTATAATGTTGTAGTAACTGCCCAT-
GCCTTTGTTATAATTTCTTCATAGTTTATACCAATTATAATTTGGTGGATTTGGTAATTGACTCGTAC-
CCCTTATATTAGGAGCACCTGATATAGCATTCCACGTATAAACAATATAAGATTCTGACTTTTACCTC-
CATCTTTACACTACTCCTATCAAGAGGAATGGTAGAAAGAGGAGTCGGTACAGGATGAACCTGT-
GTATCCCCGCTAGCAGCAGGAATGGCCACGCTGGAGCCTCAGTTGACATAGGAATTTTTCAC-
TACACCTCGCCGGAGTCTCATCAATTCTAGGTGCAGTTAATTTTATAACAACCTGTTATTAACAT-
ACGCCCTGCAGGAATAACTATAGACCGTATACCACTTTTCGTGTGAGCAGTATTTATTACAGC-
CCTACTCCTTCTTTTATCTCTACCAGTCTTAGCCGGAGCAATTACTATACTTCTAACTGACCGAAATCT-
TAACACCTCTTTCTTCGATCCAGCAGGAGGTGGAGATCCAATTCTATACCAACACTTATTT

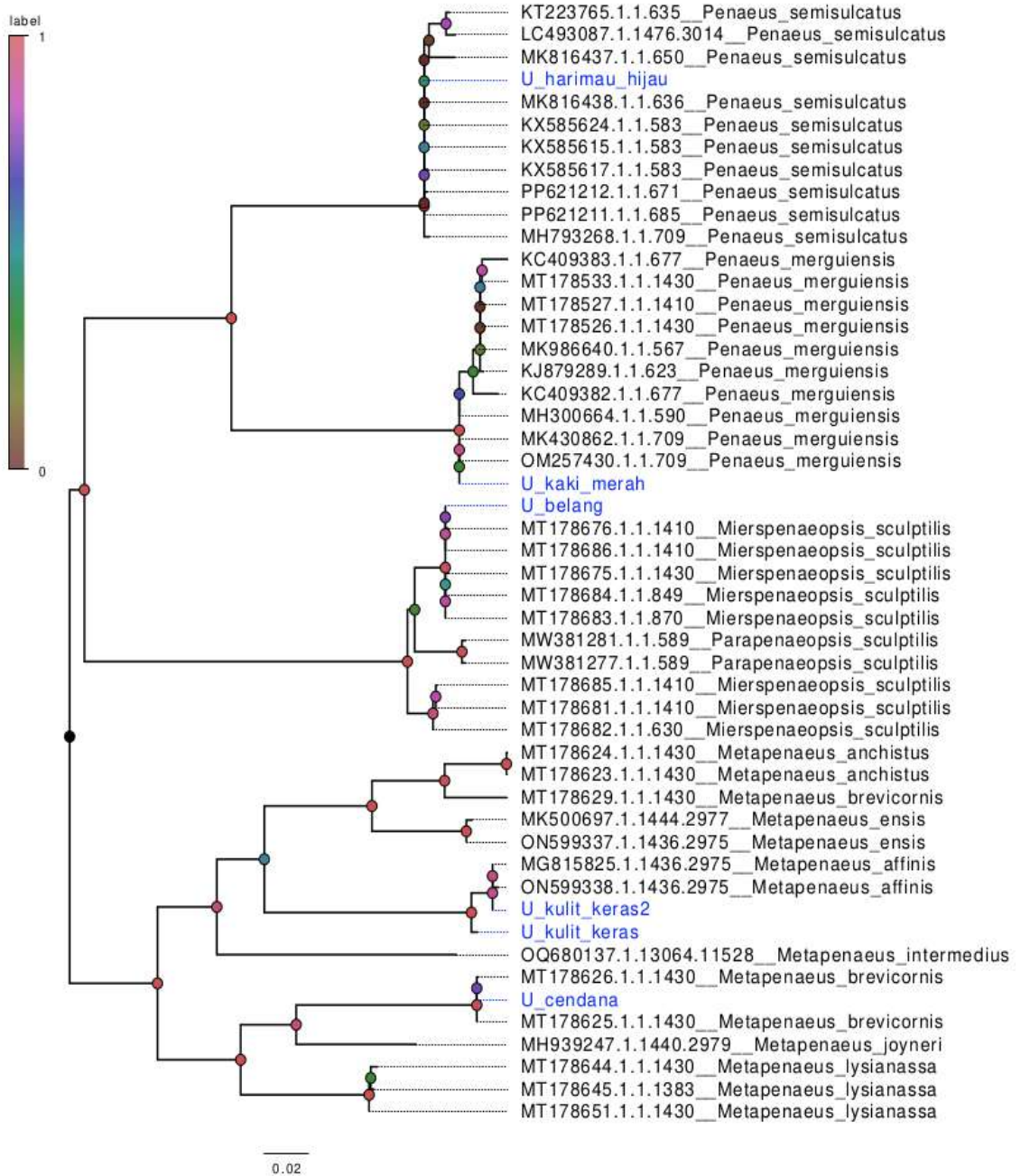


Figure 3. Phylogenetic tree constructed from the maximum likelihood tree depicting the evolutionary relationships among the shrimp samples. Nodes are color-coded to reflect bootstrap support values, providing an indication of the reliability of each branch. Branch lengths represent the number of substitutions per site, while blue taxa correspond to the sequenced samples.

Taxonomic identification

By using the MIDORI database and BLASTn with an e-value threshold of $1e-100$, the shrimp samples were identified with the matching percentage as in Table 2. Table 3 summarises the results based on the matching percentages for all samples.

Table 2. e-value threshold of 1e-100 and the coverage of the samples.

Sample ID	Accession Number	% ID	coverage
Rainbow shrimp (udang belang)	MT178686.1.1.1410__ <i>Mierspenaeopsis_sculptilis</i>	100	93.46
	MT178683.1.1.870__ <i>M. sculptilis</i>	100	93.46
	MT178675.1.1.1430__ <i>M. sculptilis</i>	100	93.46
	MT178684.1.1.849__ <i>M. sculptilis</i>	100	93.01
	MT178676.1.1.1410__ <i>M. sculptilis</i>	99.84	93.46
	MW381281.1.1.589__ <i>Parapenaeopsis_sculptilis</i>	96.68	86.93
	MW381277.1.1.589__ <i>P. sculptilis</i>	96.68	86.93
	MT178685.1.1.1410__ <i>M. sculptilis</i>	96.58	93.31
	MT178682.1.1.630__ <i>M. sculptilis</i>	96.58	93.31
	MT178681.1.1.1410__ <i>M. sculptilis</i>	96.58	93.31
Green tiger shrimp (udang harimau hijau)	PP621211.1.1.685__ <i>Penaeus_semisulcatus</i>	100	100
	MK816438.1.1.636__ <i>P. semisulcatus</i>	100	94.83
	MK816437.1.1.650__ <i>M. sculptilis</i>	100	94.83
	KX585624.1.1.583__ <i>M. sculptilis</i>	100	86.32
	PP621212.1.1.671__ <i>M. sculptilis</i>	99.85	100
	MH793268.1.1.709__ <i>M. sculptilis</i>	99.85	100
	LC493087.1.1476.3014__ <i>M. sculptilis</i>	99.85	100
	KT223765.1.1.635__ <i>M. sculptilis</i>	99.84	93.92
	KX585617.1.1.583__ <i>M. sculptilis</i>	99.82	86.32
	KX585615.1.1.583__ <i>M. sculptilis</i>	99.82	86.32
Yellow shrimp (udang cendana)	MT178626.1.1.1430__ <i>Metapenaeus_brevicornis</i>	100	93.46
	MT178625.1.1.1430__ <i>Metapenaeus_brevicornis</i>	100	93.46
	MH939247.1.1440.2979__ <i>Metapenaeus_joyneri</i>	89.67	100
	MT178651.1.1.1430__ <i>Metapenaeus_lysianassa</i>	87.13	93.31
	MT178645.1.1.1383__ <i>Metapenaeus_lysianassa</i>	86.97	93.31
	MT178644.1.1.1430__ <i>Metapenaeus_lysianassa</i>	86.97	93.31
	ON599338.1.1436.2975__ <i>Metapenaeus_affinis</i>	85.78	99.39
	MG815825.1.1436.2975__ <i>Metapenaeus_affinis</i>	85.78	99.39
	OQ680137.1.13064.11528__ <i>Metapenaeus_intermedius</i>	85.13	100.15
Banana shrimp (udang kaki merah)	ON599337.1.1436.2975__ <i>Metapenaeus_ensis</i>	84.25	99.39
	OM257430.1.1.709__ <i>Penaeus_merguensis</i>	100	100
	MT178533.1.1.1430__ <i>Penaeus_merguensis</i>	100	93.46
	MT178527.1.1.1410__ <i>Penaeus_merguensis</i>	100	93.46
	MT178526.1.1.1430__ <i>Penaeus_merguensis</i>	100	93.46
	KJ879289.1.1.623__ <i>Penaeus_merguensis</i>	100	92.71

	MH300664.1.1.590__ <i>Penaeus_merguensis</i>	100	89.66
	MK986640.1.1.567__ <i>Penaeus_merguensis</i>	100	86.17
	MK430862.1.1.709__ <i>Penaeus_merguensis</i>	99.85	100
	KC409383.1.1.677__ <i>Penaeus_merguensis</i>	99.85	98.48
	KC409382.1.1.677__ <i>Penaeus_merguensis</i>	99.85	98.48
Jinga shrimp (udang kulit keras2)	MG815825.1.1436.2975__ <i>Metapenaeus_affinis</i>	100	100
	ON599338.1.1436.2975__ <i>Metapenaeus_affinis</i>	99.54	100
	MT178651.1.1.1430__ <i>Metapenaeus_lysianassa</i>	85.83	93.31
	MT178645.1.1.1383__ <i>Metapenaeus_lysianassa</i>	85.67	93.31
	MT178644.1.1.1430__ <i>Metapenaeus_lysianassa</i>	85.67	93.31
	MT178624.1.1.1430__ <i>Metapenaeus_anchistus</i>	85.55	93.62
	MT178623.1.1.1430__ <i>Metapenaeus_anchistus</i>	85.55	93.62
	MT178626.1.1.1430__ <i>Metapenaeus_brevicornis</i>	85.39	93.62
	MT178625.1.1.1430__ <i>Metapenaeus_brevicornis</i>	85.39	93.62
	MT178629.1.1.1430__ <i>Metapenaeus_brevicornis</i>	85.20	93.46
Jinga shrimp (udang kulit keras)	MG815825.1.1436.2975__ <i>Metapenaeus_affinis</i>	98.78	100
	ON599338.1.1436.2975__ <i>Metapenaeus_affinis</i>	98.33	100
	MT178651.1.1.1430__ <i>Metapenaeus_lysianassa</i>	85.83	93.31
	MT178645.1.1.1383__ <i>Metapenaeus_lysianassa</i>	85.67	93.31
	MT178644.1.1.1430__ <i>Metapenaeus_lysianassa</i>	85.67	93.31
	MK500697.1.1444.2977__ <i>Metapenaeus_ensis</i>	85.26	100
	MT178626.1.1.1430__ <i>Metapenaeus_brevicornis</i>	85.23	93.62
	MT178625.1.1.1430__ <i>Metapenaeus_brevicornis</i>	85.23	93.62
	MT178624.1.1.1430__ <i>Metapenaeus_anchistus</i>	85.23	93.62
	MT178623.1.1.1430__ <i>Metapenaeus_anchistus</i>	85.23	93.62

Table 3. The identified species based on the COX 1 sequences.

Local name	Identified & confirmed species	Matching percentage
Udang cendana	<i>Metapenaeus_brevicornis</i>	100
Udang kulit keras	<i>Metapenaeus_affinis</i>	100
Udang belang	<i>Mierspenaeopsis_sculptilis</i>	100
Udang harimau hijau	<i>Penaeus_semisulcatus</i>	100
Udang kaki merah	<i>Penaeus_merguensis</i>	100

Mitochondrial DNA barcoding is a powerful tool for taxonomic identification, especially for non-taxonomists. Traditional morphological identification requires expert knowledge and can be complicated due to cryptic morphology, life cycle stages and phenotypic similarities that obscure distinctions (Farias et al., 2023). There will be morphological differences at each stage of the life cycle of the shrimps as well. Therefore, mitochondrial DNA barcoding gives a precise result regardless of which stage the shrimp samples are collected.

However, the accuracy of DNA barcoding depends on the quality and completeness of the reference sequences in databases like NCBI or MIDORI. Errors in the reference data could lead to misidentification (Goudey et al., 2022). This highlights the importance of continual refinement of these databases by the research community.

The method used in this study aligns with previous studies, such as Saleky et al. (2022), which successfully identified *Mierspenaeopsis sculptilis* from the coastal water of Pantai Payum Kabupaten Merauke, Indonesia. Similarly, Rajkumar et al. (2015) used the COX1 barcoding approach to identify shrimp species of *Penaeus semisulcatus*, *Metapenaeus dobsoni*, *Metapenaeus brevicornis*, *Fenneropenaeus indicus*, *Parapenaeopsis stylifera* and *Solenocera crassicornis* along the Coromandel Coast of Tamil Nadu, India. These studies reinforced the practicality and reliability of this method for species identification in aquaculture research.

From the phylogenetic analysis (Figure 2), the relationships among the species are evident. Jingga shrimp and yellow shrimp are closely related as both belong to the genus *Metapenaeus*. Similarly, green tiger shrimp and banana shrimp are grouped within the genus *Penaeus*. Udang kulit keras and udang kulit keras2 are of the same species, both of which are from the waters of Pulau Sayak but sampled at different times, based on the sequencing result against MIDORI2 library. The phylogenetic tree shows that udang kulit keras2 is more closely related to the *M. affinis* in the MIDORI2 library, as compared to udang kulit keras, which showed to have a longer branch on the phylogenetic tree.

For broodstock programs, mitochondrial DNA barcoding is essential for ensuring species accuracy, particularly in the early stages of recruitment. Misidentification can lead to unintended hybridization, compromising the integrity of breeding programs. By employing DNA barcoding alongside morphological identification, aquaculture facilities can mitigate this risk and optimize broodstock management.

Conclusion

Precise species identification using DNA barcoding of the mitochondrial cytochrome c oxidase subunit I (COX1) gene is essential in aquaculture, particularly for broodstock programs. This method complements morphological identification and is indispensable when dealing with cryptic species, indistinguishable morphologies, or when taxonomic expertise is unavailable. By ensuring accurate species identification, DNA barcoding helps prevent hybridization and supports the development of purebred lines critical to sustainable aquaculture practices. This tool should be used in tandem with traditional methods to maximize reliability and efficiency in species identification and broodstock development.

Acknowledgements

The authors wish to express their gratitude to the Director General of the Department of Fisheries Malaysia for granting permission to publish this article. We also acknowledge the invaluable contributions of our colleagues and staff at the Fisheries Research Institute (FRI) Pulau Sayak, Kedah, who were directly or indirectly involved in this research.

Special thanks are extended to Nazariah Mohd Nazri for her assistance in sample preparation for analysis. We are also deeply grateful to the Senior Director of Research, Dr. Azhar Hamzah and the Director of IPP Pulau Sayak, Mohammed Suhaimie Abd Manaf, for their unwavering support and encouragement.

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Emergence of *Alexandrium minutum* and high-level PSP toxins in green mussels (*Perna viridis*): A case study from Port Dickson, Malaysia

MOHD NOR AZMAN AYUB^{1*}, WAN NORHANA MD NOORDIN¹, ROZIAWATI MOHD RAZALI¹, ZULIFAH ROHANI²

¹ Fisheries Research Institute, Department of Fisheries Malaysia, Batu Maung, 11960, Penang, Malaysia

² Fisheries Biosecurity Laboratory Centre, No. 82, Jalan Carruthers of Jalan Sultan Salahuddin, 50480 Kuala Lumpur, Malaysia

*Corresponding author: nor_azman@dof.gov.my

Abstract: On 1st April 2024, a case of paralytic shellfish poisoning (PSP) was reported to the Department of Fisheries (DOF) Malaysia following a notification of eight suspected food poisoning cases in Port Dickson, Negeri Sembilan. All cases were linked to the consumption of mussels, with two individuals admitted to the Intensive Care Unit (ICU) after experiencing paralysis. Green mussel (*Perna viridis*) and seawater samples were collected on 2nd April 2024 for toxin and causative organism detection. Initial PSP toxin screening was conducted using enzyme-linked immunosorbent assay (ELISA), followed by confirmation via high-performance liquid chromatography (HPLC). Investigations identified PSP toxin as the causative agent, with recorded concentrations reaching 82,400.66 $\mu\text{g kg}^{-1}$ —103 times above the safety limit of 800 $\mu\text{g STX eq kg}^{-1}$ tissue (or 800 ppb). The PSP toxin profile was dominated by gonyautoxin groups, GTX1&4, followed by GTX2&3, GTX5, and saxitoxin (STX). Microscopic examination of seawater samples confirmed the presence of toxic dinoflagellates from the genus *Alexandrium*, with *Alexandrium minutum* later confirmed as the causative species based on toxin profiling and organism identification. Consequently, the DOF issued a ban on the collection and sale of shellfish from Port Dickson and surrounding waters. The ban was lifted after four months, once toxin levels in shellfish dropped below 800 ppb and *Alexandrium* cell counts were no longer detected in three consecutive samplings.

Keywords: *Alexandrium minutum*, PSP, shellfish, saxitoxin, ELISA, HPLC

Abstrak: Pada 1 April 2024, satu insiden keracunan kerang-kerangan paralitik (PSP) telah dilaporkan kepada Jabatan Perikanan (DOF) Malaysia, berikutan pemberitahuan mengenai lapan kes disyaki mengalami keracunan makanan di Port Dickson, Negeri Sembilan. Semua kes dikaitkan dengan pengambilan kupang, dengan dua individu dimasukkan ke unit rawatan rapi (ICU) setelah mengalami lumpuh. Sampel kupang (*Perna viridis*) dan air laut telah diambil untuk pengesanan toksin dan organisma penyebab pada 2 April 2024. Saringan awal toksin PSP dilakukan menggunakan ujian imunosorben enzim (ELISA) dan kemudiannya disahkan melalui kromatografi cecair prestasi tinggi (HPLC). Hasil siasatan mengenal pasti toksin PSP sebagai agen penyebab dengan kepekatan toksin yang direkodkan sehingga 82,400.66 $\mu\text{g kg}^{-1}$, iaitu 103 kali ganda lebih tinggi daripada had keselamatan iaitu 800 $\mu\text{g STX eq kg}^{-1}$ tisu (atau 800 ppb). Profile toksin PSP menunjukkan kumpulan toksin utama ialah daripada kumpula Gonyautoxin, GTX1&4, diikuti oleh GTX2&3, GTX5, dan Saxitoxin (STX). Pemeriksaan mikroskopik terhadap sampel air laut mengesahkan kewujudan dinoflagelat toksik daripada genus *Alexandrium* yang berkemungkinan besar menjadi punca keracunan. Pencegaman lanjut dan profil toksin yang diperhatikan mengesahkan *Alexandrium minutum* sebagai organisma penyebab. Sehubungan dengan ini, DOF telah mengeluarkan notis larangan terhadap pengumpulan dan penjualan kerang-kerangan dari Port Dickson dan perairan sekitarnya. Larangan dianagkat selepas

4 bulan setelah paras toksin dalam kerangan menjadi kurang daripada 800 ppb serta hitungan sel *Alexandrium* tidak lagi dikesan dalam tiga persampelan yang berturutan.

Introduction

Paralytic shellfish poisoning (PSP) in fish, birds and mammals including humans is caused by eating filter feeders contaminated by paralytic shellfish toxin from algae (Wang, 2008). PSP toxins are mainly composed of saxitoxin (STX) and gonyautoxin (GTX), which are a group of potent neurotoxins that cause reversible binding of sodium and calcium channels with prolongation of the gating of potassium channels; this causes respiratory paralysis and can stop the heart (Cestele and Catterall, 2000). This toxin cannot be destroyed by cooking or freezing. Toxin levels contained in a single shellfish can be fatal to humans, and shellfish in the same area can contain widely variable levels of toxin.

PSP is one of the main marine toxin diseases which is characterized by high fatality and a wide distribution around the world (Halstead and Schantz, 1984). The highest number of PSP cases (2124) and deaths (120) was recorded from 1983 to 2002 in the Philippines (Ching et al., 2015). The main producers of the PSP toxins are dinoflagellates of the genera *Alexandrium*, *Pyrodinium* and *Gymnodinium* (Oshima et al., 1993; Lefebvre et al., 2008; Usup et al., 2012). STX and its 57 analogs have been identified and grouped into four subgroups: carbamate, N-sulfo-carbamoyl, decarbamoyl, and hydroxylated saxitoxins (EFSA, 2009; Wiese et al., 2010).

In Malaysia, PSP is quite common in Sabah compared to Peninsular Malaysia. The first intoxication case was recorded in 1976, involving 22 people and seven deaths on the west coast of Sabah and was associated with *Pyrodinium bahamense* var. *compressum* (Roy, 1977). Later, more PSP cases with fatalities were reported in Sabah and Labuan (Ting & Wong, 1989; Suleiman et al., 2017). Meanwhile, the first documented PSP intoxication case in the Peninsular Malaysia was disclosed in 1991 at Sebatu, Melaka, on the west coast where three people were hospitalized after eating contaminated green mussels (Usup et al., 2002). The species of concerned here was *Alexandrium tamiyavanichii*. A PSP intoxication case again occurred at Sg Geting, Tumpat, Kelantan in September 2001 due to *Alexandrium minutum* bloom which led to one death and 6 others hospitalised (Lim et al., 2004). Besides Melaka and Kelantan, ten cases with typical symptoms of PSP intoxication from consumption of oysters contaminated with *A. tamiyavanichii* at Kuantan, Pahang were reported in November 2013 and recurred in August 2014 (Mohammad-Noor et al., 2017).

On 1st April 2024, a case of PSP associated with the consumption of green mussels was reported to the Port Dickson District Health Office in Negeri Sembilan. According to the media, a total of eight food poisoning cases were linked to the ingestion of green mussels (*Perna viridis*), locally known as kupang or siput sudu (New Straits Times, 2024). Two individuals required admission to the Intensive Care Unit (ICU), while three others were hospitalized in the general ward of Port Dickson District Hospital. Five patients were subsequently warded for treatment, and one case received outpatient care. All of the victims claimed to have headaches, numbness in the hands and feet, and muscle weakness after consuming green mussels. Two victims who were admitted to the ICU experienced worse symptoms, namely paralysis. Preliminary investigation indicates that all cases comprise of three different families with similar histories of consuming green mussels brought from the Pasir Panjang fishermen market and Port Dickson public market.

In response to the incident, the Department of Fisheries, Malaysia issued a formal notice prohibiting the public from harvesting and selling mussels from Port Dickson and its surrounding waters. This article outlines key findings from the investigation into the paralytic shellfish poisoning (PSP) cases reported in Port Dickson, Negeri Sembilan.

Materials and Methods

Samples receiving and preparation

On 2nd April 2024, the Fisheries Biosecurity Laboratory, Department of Fisheries (DOF) Malaysia, Kuala Lumpur and the Marine Biotoxin Laboratory at the Fisheries Research Institute (FRI), Batu Maung, Penang received two green mussel (*Perna viridis*) samples (commercial size, 15.0 pcs bag⁻¹) from the Negeri Sembilan State Biosecurity Unit. These samples were collected from shellfish cultivation sites at Kampung (Kg.) Telok (2°24' 49.0'N, 101°56' 23.6'E) and Kampung Sungai (Sg.) Sekawang (2°24' 36.9'N, 101°55' 31.7'E) in the Pasir Panjang District, Port Dickson, Negeri Sembilan. The sites are operated by the Port Dickson Fisherman Association, which supplies fresh mussels to local markets (Figure 1). The mussel samples were washed with clean water, stored in a portable ice-insulated box, and transported to the laboratory within 24 hours. Additionally, seawater was sampled and collected from Kg. Telok and Kg. Sg. Sekawang to check for toxic microalgae species.

Later, the FRI lab also received another batch of mussel samples for PSP confirmation and seawater samples for microalgae identification. This time, a total of five samples were received from Kg. Telok, Kg. Sg. Sekawang, Port Dickson wet market, Kg. Telok fisherman market and hospital. Meanwhile, the Fisheries Biosecurity Laboratory, Kuala Lumpur continued to collect shellfish samples from Port Dickson and surrounding areas and monitor the PSP levels until July 2024.

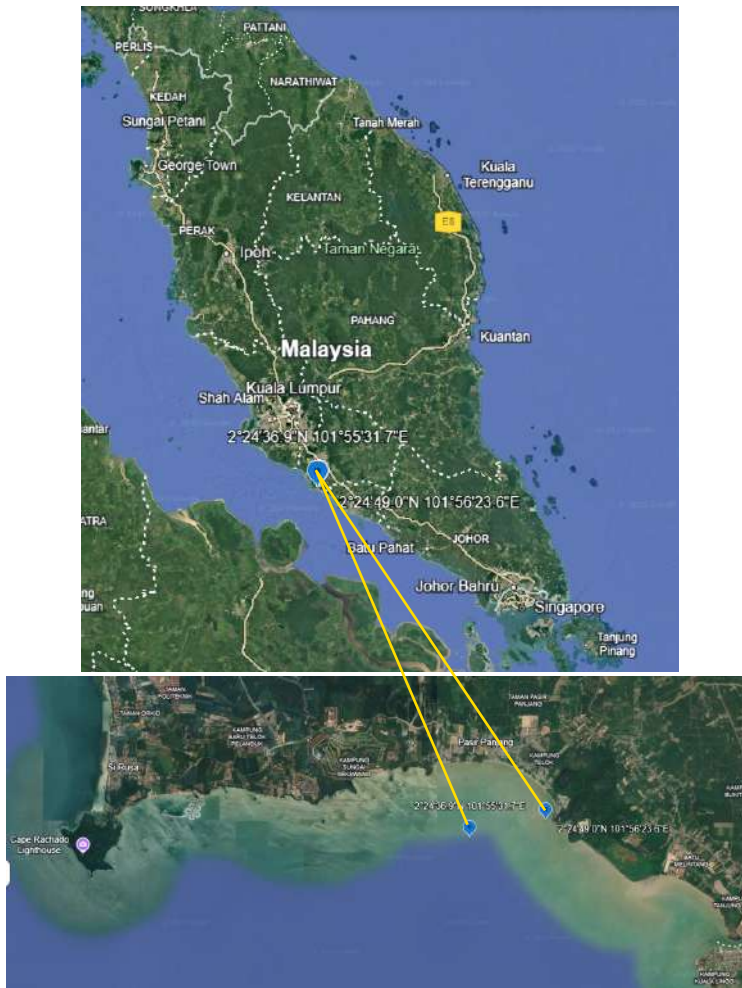


Figure 1: Google map of sample collection from Kampung Sungai Sekawang (2°24' 36.9'N, 101°55' 31.7'E) and Kampung Telok (2°24' 49.0'N, 101°56' 23.6'E) (Sources: Google Earth, 2025).

Microscopic examination of phytoplankton of sea water samples

Phytoplankton examination was carried out at two labs i.e Fisheries Biosecurity Laboratory, Kuala Lumpur (using an inverted microscope (CKX53 Olympus, Japan) and the Microalgae Lab at the Fisheries Research Institute (FRI), Batu Maung (using IX70, Olympus, Japan). Digital images were captured with a CCD camera and Analysis® software (Soft Imaging System Inc., USA). For microalgae quantification, 1 L of seawater was filtered through a 20 µm mesh sieve to concentrate the sample, which was then preserved in Lugol's iodine solution. A 1 mL sub-sample of the concentrate was loaded into a Sedgewick-Rafter counting chamber, and phytoplankton cell density (L⁻¹) was determined under a microscope in triplicate. Microalgae identification followed the *Manual on Red Tide Taxonomy of Phytoplankton and Harmful Algal Bloom Organisms*, ASEAN–Canada Cooperative Programme on Marine Science, Backer et al. (2003) and Tokio et al. (2012).

The identification of *Alexandrium* species was mainly based on the morphology of specific thecal plates, including the posterior sulcal plate (S.p), the second antapical plate (2''), the first apical plate (1'), the anterior sulcal plate (S.a), the third apical plate (3'), the sixth precingular plate (6''), and

the apical pore complex (Po). Additional distinguishing features considered were the presence and precise position of the ventral pore (v.p). The identification criteria followed the descriptions of Usup et al. (2002) and Lim et al. (2003).

Micrographs of each *Alexandrium* species were captured using a cooled CCD camera attached to an Olympus IX51 inverted research microscope (Olympus, Tokyo, Japan). The digital images were processed and analysed with Analysis® software (Soft Imaging System Inc., USA). For epifluorescence microscopy, fixed samples were stained with 1 % calcofluor white (Fluka, Japan) and examined under ultraviolet illumination using a UV filter set, following the method described in Lim et al. (2007).

PSP screening using Enzyme Immunoassay method

The Fisheries Biosecurity Laboratory, Kuala Lumpur carried out PSP screening using the EuroProxima Saxitoxin ELISA (5191SAXI, R-Biopharm AG, Germany) which is a competitive enzyme immunoassay based on antibodies directed against saxitoxin. The samples were prepared according to the manufacturer's instructions.

For sample preparation, 1.0 g of shellfish meat was homogenized in 5.0 mL of sodium acetate buffer (0.2 M, pH 5.0). The mixture was vortexed for one minute and then centrifuged at $3000 \times g$ for 10 minutes at room temperature. A total of 100 μL of the upper aqueous phase was transferred into a coded 15 mL tube, followed by the addition of 4900 μL of dilution buffer ($1 \times$ concentrate). A total of 50 μL of the prepared solution was used per well of the ELISA plate. Test solutions, conjugate, dilution buffer, antibody, and rinsing buffer were prepared according to the manufacturer's instructions. After dispensing all samples into the microtiter plate wells, the ELISA plate was sealed and shaken for one minute. It was then incubated at room temperature ($20\text{--}25^\circ\text{C}$) for 30 minutes. Following incubation, the solution was discarded, and the plate was washed three times with rinsing buffer. Subsequently, 100 μL of substrate solution was added to each well, mixed thoroughly, and incubated in the dark at room temperature ($20\text{--}25^\circ\text{C}$) for 15 minutes. To stop the reaction, 100 μL of stop solution was added to each well, and the plate was shaken well. Finally, absorbance values were read immediately at 450 nm. The toxicity of shellfish was expressed as $\mu\text{g kg}^{-1}$ tissue.

PSP confirmation using High Performance Liquid Chromatography

PSP extraction

PSP toxins were extracted using an acid extraction method with slight modification described in AOAC (2006). About 5.00 ± 0.01 grams of shellfish tissue were homogenized with 1 % acetic acid at room temperature. The mixture was then heated in a 100°C water bath for 5 min, cooled under running water, and centrifuged at 4,000 rpm, 4°C for 15 mins. The supernatant was filtered using a 0.2 μm nylon membrane, transferred to a 1.0 ml vial, and stored at -20°C until it was ready for analysis.

PSP analysis

Extracted PSP was analysed using High Performance Liquid Chromatography (HPLC) (Shimadzu, Japan) with a Pickering post-column fluorescence detector utilising the isocratic, post-column derivatization with slight modifications from Oshima (1995). Solvents were HPLC and all chemicals were analytical grade. HPLC condition used for this analysis is summarized in Table 1.

Table 1: HPLC conditions used for PSP analysis (Oshima,1995).

Parameter	Condition
Columns	Luna C18(2) column (150 x 4.6 mm inner diameter, 120 Å, 5 µm) (Phenomenex, Torrance, USA) with a security guard cartridge (C18, 4 x 3.0 mm inner diameter) (Phenomenex, Torrance, USA)
Mobile phase	GTX group (GTX1-GTX5) 2 mM heptanesulfonate in 10 mM ammonium phosphate buffer containing 1 % acetonitrile STX group (dcSTX, STX) 2 mM heptanesulfonate in 30 mM ammonium phosphate buffer containing 5 % acetonitrile
Injection volume	10 µl
Flow rate	0.8 ml min ⁻¹
Temperature	27 °C, the post-column temperature was set at 85 °C for all runs
Acidifying reagent	0.5 M acetic acid
Flow rate	0.4 ml min ⁻¹
Oxidizing reagent	7 mM periodic acid in 50 mM potassium phosphate buffer, pH 9.0
Flow rate	0.4 ml min ⁻¹
Detection	
Excitation	330 nm
Emission	395 nm

All samples were analysed in triplicate. GTXs (GTX 1-5) and STXs (STX, dcSTX) were analysed separately. Toxin identification and quantification were carried out by comparison with standard toxin materials. The concentrations of each toxin or epimeric pair (GTX1&4, GTX2&3, GTX5, STX and dcSTX) were calculated with linear calibration curves, achieved using PSP certified reference standards. For the calculation of toxicity from HPLC chromatograms, the following values of toxicity factor convert to STX_{equiv.} were determined: GTX1 (0.99), GTX2 (0.36), GTX3 (0.64), GTX4 (0.73), GTX5 (0.06), C1 (0.01), C2 (0.1), dcSTX (0.51), NEO (0.92) and STX (1). To avoid mistaking false peaks as toxins, the samples were re-analysed with the same HPLC with fluorescence online detection (FLD) procedure, but replaced the oxidizing reagent by distilled water. Although oxidation doesn't take place under these conditions, some false peaks can still be observed (Lagos et al., 1999). Toxicity of shellfish was expressed as µg 100 g⁻¹ tissue. For standardisation purpose, the toxicity values from HPLC were multiply by ten for easy comparison with the ELISA reading and ESFA (2009).

Certified reference materials were purchased from the National Research Council of Canada, Halifax, Nova Scotia, Canada: Saxitoxin (STX), decarbamoylsaxitoxin (dcSTX), gonyautoxin-5 (GTX5), gonyautoxin-1&4 (GTX1&4), gonyautoxin-2&3 (GTX2&3). Calibration solutions were diluted to prepare the calibration standards curve, using 0.03 M acetic acid as diluent. Two mixtures of toxin standards were prepared, one mixture contained dcSTX and STX, and another contained GTX1&1, GTX2&3 and GTX5.

Results and Discussion

Examination of seawater samples collected from the affected areas revealed the presence of several toxic microalgae species potentially responsible for the shellfish poisoning incident. Other microalgae species noted at normal counts were *Amphiprora* sp., *Thalassiosira* sp., *Skeletonema* sp., *Ditylum* sp., *Rhizosolenia* sp., *Corethron* sp., *Cheatoceros* sp., *Cylindrotheca* sp., *Thalassionema*

sp., *Odontella* sp., *Coscinodiscus* sp., *Lauderia* sp., *Guinardia* sp., *Tripos furca*, *Leptocylindricus* sp. and several others. Both sampling locations showed low cell densities of known producers of harmful toxins i.e *Alexandrium* sp. with some samples exceeding 1,000 cells L⁻¹ (Table 2). Notably, the cell densities of *Alexandrium* sp. at Kg. Telok was slightly higher than at Kg. Sg. Sekawang. A toxic microalga, *Alexandrium* sp. was only noted on the samples collected on 2nd April 2024. Another toxic microalga, *Pseudo-nitzschia* sp. was also noted in the seawater samples. *Pseudo-nitzschia* sp. are the producer of Domoic acid (DA) and other toxic DA isomers which are responsible for Amnesic Shellfish Poisoning. Continuous monitoring by the Kuala Lumpur Fisheries Biosecurity Centre over eight consecutive weeks consistently recorded either no presence or only few cells of these toxic microalgae. These observations suggest that the harmful algal bloom may have occurred earlier than April and had already subsided by the time poisoning cases on the 1st of April 2024.

Table 2: Cell density (cells L⁻¹) of main potentially toxic phytoplankton species observed in the sweater samples from the affected areas in Port Dickson.

Date (2024)	Toxic Species	Microalgae Lab, Fisheries Research Institute, Batu Maung		Fisheries Biosecurity Laboratory, Kuala Lumpur	
		Kg. Telok	Kg. Sg. Sekawang	Kg. Telok	Kg. Sg. Sekawang
(cells L ⁻¹)					
2 nd April	<i>Pseudo-nitzschia</i> sp.	150-400	200-250	400	2,600
	<i>Alexandrium</i> sp.	660-1,550	400-1,000	3,400	300
3 rd April	<i>Pseudo-nitzschia</i> sp.	550-3,875	1,350-11,175	17,600	1,100
	<i>Alexandrium</i> sp.	-	-	400	-

The detailed morphological examination of the thecal plates of the *Alexandrium* samples from Kg Telok, Port Dickson revealed the species as *A. minutum* (Figure 2). The cells are generally oval in shape, with a deeply indented cingulum. The first apical plate (1') is of moderate width, slightly curved left and right margins. This plate makes direct contact with the apical pore (Po). The apical pore is positioned centrally within the complex. A ventral pore (v.p) is clearly visible, positioned about halfway along the right margin of 1'. The anterior sulcal plate (s.a) is longer than wide. The posterior sulcal plate (s.p) is distinctly wider than long. The sixth precingular plate (6'') is notably narrow and elongated, a key diagnostic feature for this species. The third apical plate (3') is asymmetrical, with the left anterior margin longer than the right. Other distinguishing characteristics include the rhomboid shape of the left anterior lateral sulcal plate (s.s.a.) and the short length of the left posterior lateral sulcal plate (s.s.p.) within the sulcus. These morphological traits are consistent with descriptions provided by Usup et al. (2002) and Lim et al. (2003) confirming the identification of the bloom-forming species as *A. minutum*.

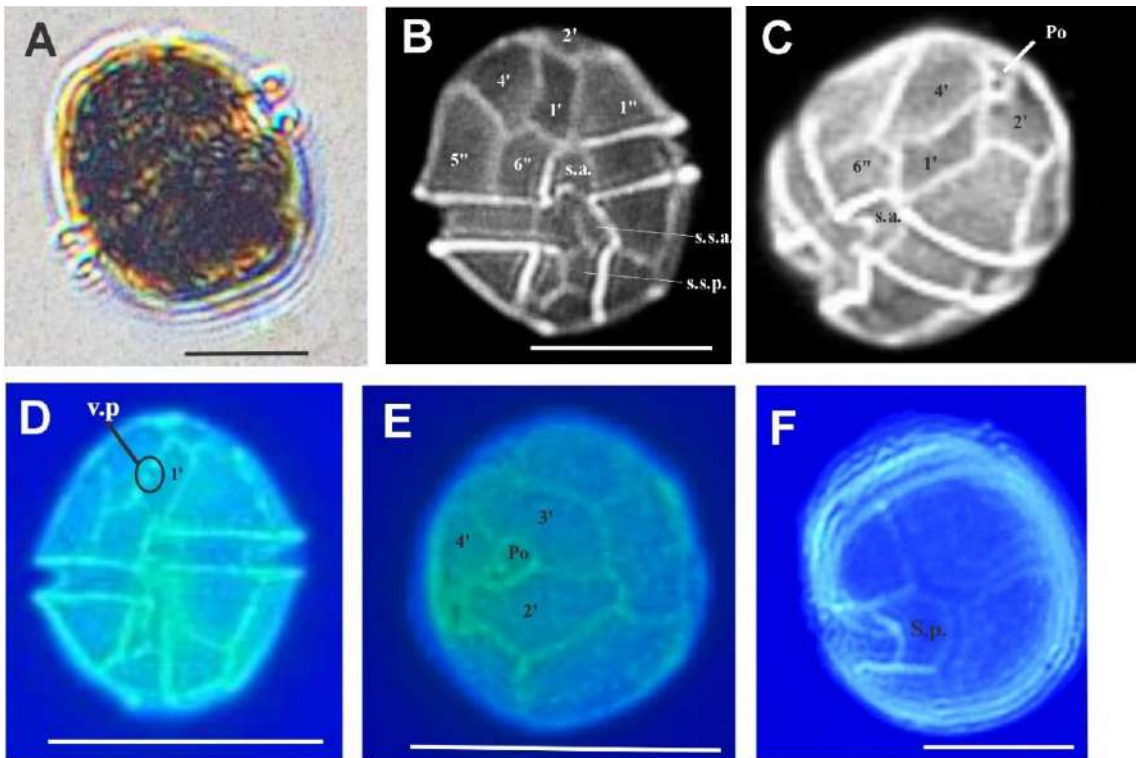


Figure 2: *Alexandrium minutum*: (A) a vegetative cell; (B) ventral view showing the first apical plate (1'), the second apical plate (2'), the fourth apical plate (4'), the first precingular plate (1''), the tall sixth precingular plate (6''), the fifth precingular plate (5''), the wide anterior sulcal plate (s.a.), the left anterior lateral plate (s.s.a.) and left posterior sulcal (s.s.p.); (C) apical view showing the 1', 2' and 4' touches an apical pore (Po); (D) the ventral pore (v.p) (rounded) is in the midway of 1'; (E) the apical view showing the 2', 3', and 4' touches Po; (F) the antapical view showing the wide posterior sulcal plate (s.p.). Scale bar (A) – (F) 10µm.

The investigation conclusively identified paralytic shellfish poisoning (PSP) toxins as the causative agent in the Port Dickson shellfish poisoning incident. The results of PSP analysis from samples received on the 2nd of April 2024 are presented in Table 3. The recommended safety limit of 800 µg kg⁻¹ (ppb) PSP toxins (saxitoxin and analogues) in total quantity (measured in the whole body or any part edible separately) as stated in the European Union, Regulation (EC) No 853/2004, is used as reference in this case (EFSA, 2009). Paralytic shellfish poisoning (PSP) is relatively common in Malaysia, particularly in the state of Sabah (Roy, 1977; Ting & Wong, 1989; Suleiman et al., 2017). Within Peninsular Malaysia, the recent incident in Port Dickson, Negeri Sembilan represents the fourth reported PSP case, following previous occurrences in Sebatu, Melaka in 1991, caused by *Alexandrium tamiyavanichii* (Usup et al., 2002); in Tumpat, Kelantan, attributed to *A. minutum* (Lim et al., 2004; Wan Norhana et al., 2005); and in Kuantan, Pahang, also linked to *A. tamiyavanichii* (Mohammad-Noor et al., 2017).

In general, PSP results from the ELISA method were generally lower compared to the HPLC. This is logical as ELISA is considered as semi qualitative screening method for measuring shellfish toxicity compared to HPLC. HPLC method could provide more accurate and detailed toxin profiles allowing for the identification and quantification of individual PSP toxins. The results revealed that

all green mussel samples received on 2nd April 2024 contained PSP toxin with levels exceeding the permissible limit of 800 µg kg⁻¹ as indicated in Table 3. The mussel samples from Kg. Telok showed very high PSP toxin content (82,400.66 µg kg⁻¹) which is about 99-107 times more than the permissible limits. On the other hand, PSP levels in samples from Kg. Sg. Sekawang recorded reading of 8,930.63 µg kg⁻¹. Although lower than those observed from Kg. Telok, it is still about 8.2-14 times more than the permissible limit.

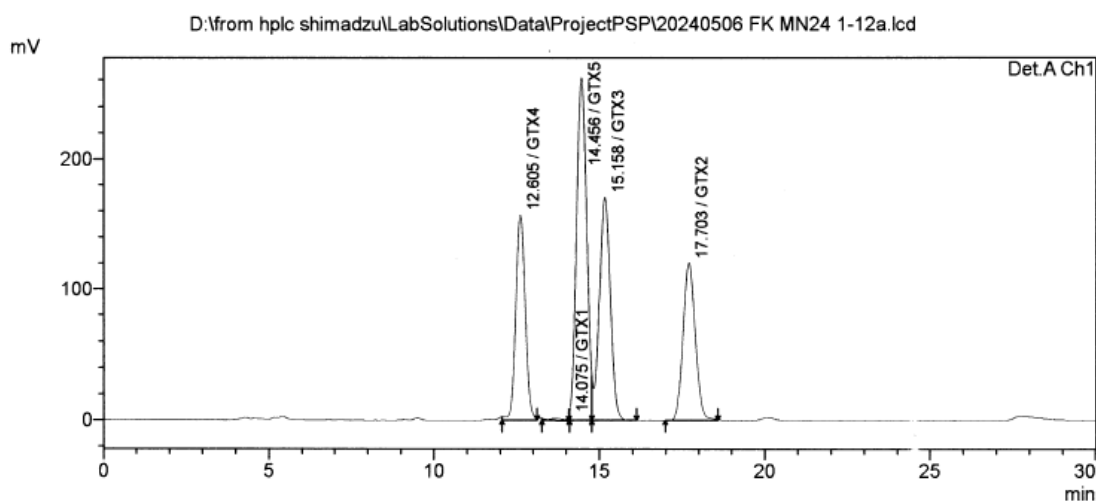
Table 3: PSP levels detected in the green mussel samples from two stations at Port Dickson, Negeri Sembilan.

Sampling locations	(µg kg ⁻¹)	
	ELISA	HPLC
Kg. Telok	10,046.10	82,400.66
Kg Sg. Sekawang	4,960.21	8,930.63

HPLC analysis revealed that the highest PSP toxin groups in all samples belonged to the GTX1 & 4 group, followed by GTX2 & 3, GTX5, and lastly STX (Table 4). The chromatogram profiles of the saxitoxin group detected in the standard solution and mussel samples were depicted in Figure 3a and 3b, respectively. GTX1&4 group in samples from Kg. Telok constituted slightly more than 90.6 % of the toxicity, GTX2&3 constituted about 0.02-8.5% while GTX5 and STX group constituted less than 1 %. A nearly identical toxin distribution pattern was observed in samples from Kg. Sg. Sekawang.

Table 4: PSP toxin profiles in the green mussel samples from two stations at Port Dickson, Negeri Sembilan.

Location	Concentrations (ug kg ⁻¹)							
	GTX4	GTX1	GTX5	GTX3	GTX2	dcSTX	STX	Total
Kg. Telok	37,100.49	37,510.87	450.57	7,001.16	160.78	0.00	140.79	82,400.66
Kg. Sg. Sekawang	4,200.44	2,690.62	190.00	1,730.59	90.06	19.00	0.00	8,930.63



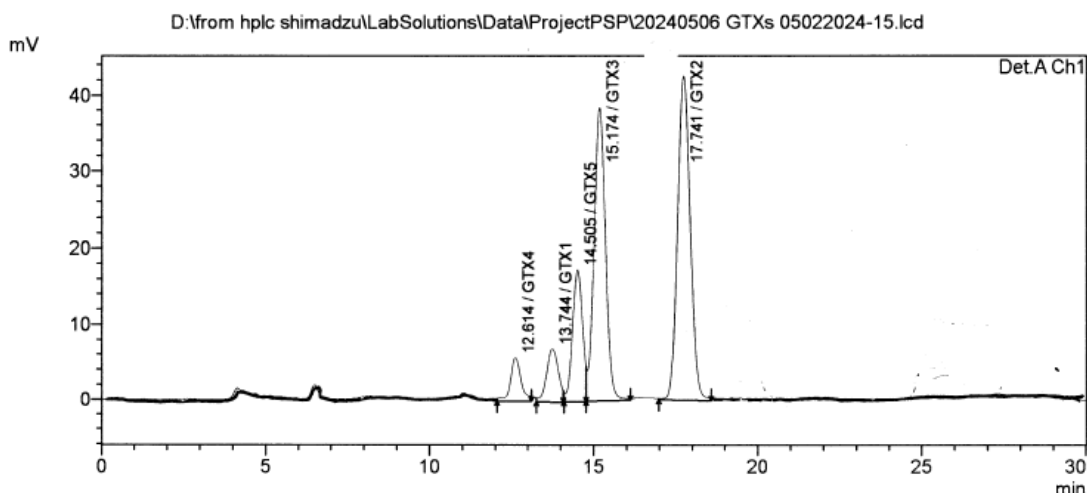


Figure 3: Chromatogram profiles of (a) samples from Kg Telok, Port Dickson and (b) GTXs standard reference material.

The second batch of samples received by the FRI lab the following week also indicate mussel samples with PSP levels exceeding permissible levels. Figure 4 shows the quantity and profiles of PSP toxins in five mussel samples analysed. Mussel samples taken from Kg. Telok fisherman market, Kg Telok mussel cultivation area and hospital exhibited higher PSP toxin contents than samples from Kg. Sg. Sekawang and Port Dickson wet market. These findings suggest that mussel from Kg. Telok might be the cause of the poisoning cases in Port Dickson.

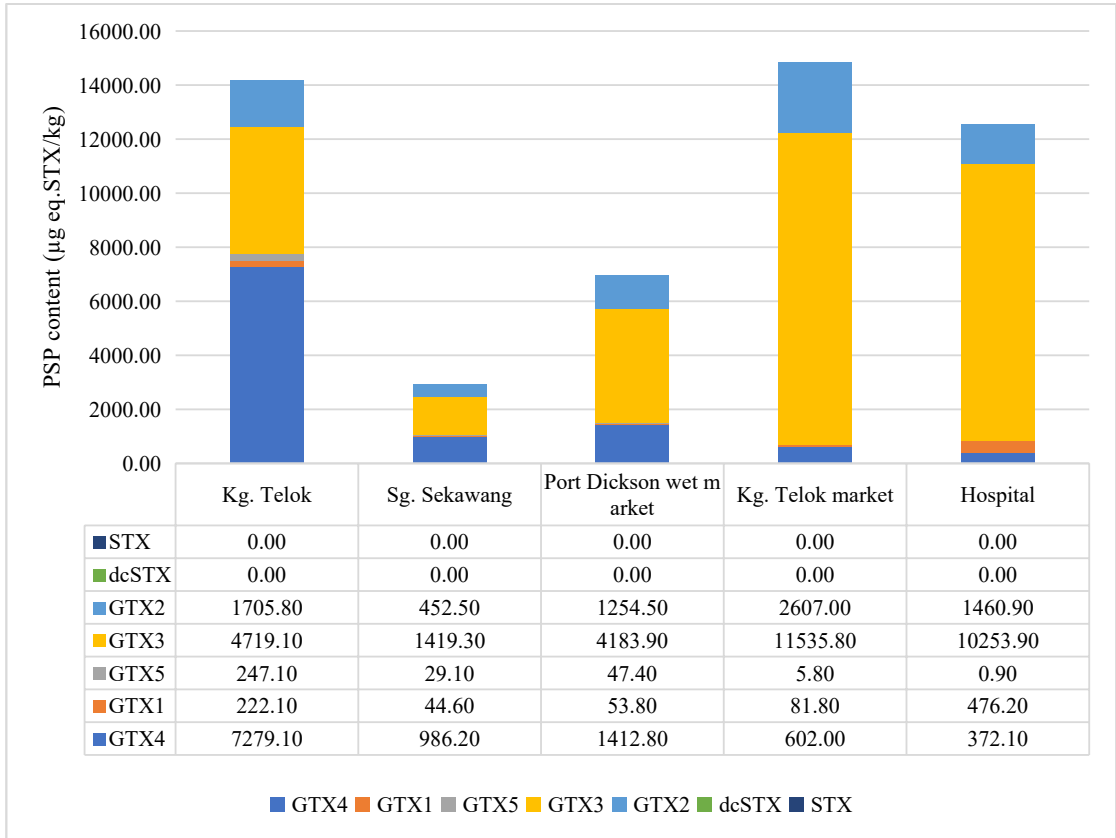


Figure 4: PSP contents and profiles in mussels from five different sources.

Similarly, GTX2&3 and GTX1&4 groups were the main toxic constituents followed by groups GTX2&3 and GTX5. STX and dcSTX groups were not detected in any of the mussel samples. GTX1, GTX2, GTX3, and GTX4 were reported as the common profile of *Alexandrium minutum* (Usup et al., 2006)

The green mussels' samples collected in May, June and July 2024 generally show a gradual decrease of PSP toxins. The PSP levels recorded in May 2024 were between 72.49 to 935.00 $\mu\text{g kg}^{-1}$ and reduced to 7.86 to 675.95 $\mu\text{g kg}^{-1}$ in June 2025. In July 2024, the readings were only between 46.69 to 115.2 $\mu\text{g kg}^{-1}$ which are way below the recommended safety limit. As clearly shown in Figure 1, the mussel cultivation area in Kg. Sg. Sekawang faced the open waters along the Straits of Melaka, while the one in Kg. Telok (Bay in Bahasa Melayu), as the name implies, is situated along a semi-enclosed bay with subdued tidal currents, creating a low-flushing environment. Due to the area's geographical characteristics, the movement of toxic microalgae is likely restricted, resulting in greater toxin accumulation in shellfish through their filter-feeding activities. Furthermore, the flushing of these toxins from shellfish may also take longer under such conditions. As a result, fluctuations in toxic microalgae cell counts and PSP toxin levels were consistently observed in our findings. Continuous monitoring is therefore essential, as elevated PSP concentrations can persist in shellfish for extended periods following a harmful algal bloom. This was evidenced in a case reported in Kuala Penyu, Sabah, where high PSP levels (490 $\mu\text{g 100 g}^{-1}$) were still detected in shellfish samples even six months after the bloom event (Suleiman et al., 2017).

The presence of *Alexandrium* species in the coastal waters of Peninsular Malaysia is not new. At least five species of *Alexandrium* have been recorded namely *A. affine*, *A. leei*, *A. minutum*, *A. tamarensense* and *A. tamiyavanichii* (Usup et al., 2002). A bloom of *A. minutum* was first recorded in 2001 in a coastal lagoon at Sg. Geting, Tumpat, Kelantan, and was associated with PSP cases that resulted in six hospitalizations and one fatality following the consumption of contaminated clams (Lim et al., 2004, Wan Norhana et al., 2005). This species reoccurred during another bloom event in 2015, leading to the detection of high saxitoxin concentrations in clams and the subsequent enforcement of a shellfish harvesting and sales ban in the affected area (Lau et al. 2017). Beyond *Alexandrium*, PSP toxins are known to be produced by over 20 species of marine dinoflagellates. In Malaysia, at least seven PSP-producing species have been previously documented: *A. minutum*, *A. taylori*, *A. peruvianum*, *A. tamarensense*, *A. tamiyavanichii*, *Gymnodinium catenatum* and *Pyrodinium bahamense* var. *compressum* (Usup et al., 2002; Lim et al., 2012, Yurimoto et al., 2012). Another toxic microalga, *Pseudo-nitzschia* sp. was also noted in the seawater samples. However, it is not the toxin producer in this incident. *Pseudo-nitzschia* sp. are the producer of Domoic acid (DA) and other toxic DA isomers which are responsible for Amnesic Shellfish Poisoning.

Our findings strongly suggest that the species responsible for the poisoning cases in Port Dickson is *A. minutum* based on the morphological of the thecal plates and the toxin profiles. By August 2024, the Department of Fisheries (DOF) officially lifted the ban on the harvesting and sale of shellfish, including mussels, in the waters of Port Dickson and Melaka. This decision followed a consistent decline in PSP toxin levels in mussel samples to values below the recommended safety threshold of 800 ppb, observed over several consecutive weeks from May to July 2024. The announcement was formally made on 12 August 2024, as reported by The Star (2024).

Conclusion

The toxin profiles and the co-occurrence of *A. minutum* confirmed the species responsible for the poisoning cases in Port Dickson. The algae bloom involving *A. minutum* could have happened earlier than April. The exact location of the source of contamination is at the cultivation area in Kg Teluk, Pasir Panjang, Port Dickson.

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Captive breeding of clownfish: Potential and future

ELEANOR DANIELLA LOKMAN*, KAHARUDIN BIN MD SALLEH, DZULFIKKAR BAITUL MA'MUR

¹*Fisheries Research Institute (FRI) Batu Maung, 11960 Pulau Pinang, Malaysia*

*Corresponding author: eleanor@dof.gov.my

Abstract: The clownfish (*Amphiprion ocellaris*), also known as the false percula, or ikan Inggü in Bahasa Melayu is a popular marine ornamental species valued for its bright colours and resilience. This study focuses on the captive breeding and hybridization of clownfish to reduce pressure on wild populations and support conservation. From 2018 to 2023, ornamental fish like goldfish, bettas, and clownfish significantly contributed to Malaysia's export economy, with 2022 production reaching 200 million fish and MYR 453 million in revenue. Due to rising demand, overharvesting has become a threat to wild stocks. This research, conducted at the Fisheries Research Institute in Batu Maung, tested breeding methods using wild-caught and store-bought clownfish in both indoor and outdoor nurseries. Findings highlight the importance of managing genetic diversity and disease risks. The study covers breeding techniques, hybridization, ethical considerations, and conservation impact—emphasizing that responsible breeding practices, backed by science and collaboration, are key to protecting biodiversity while sustaining the ornamental fish industry

Keywords: Clownfish, Captive breeding, Marine ornamental, Hybridization, Conservation.

Abstrak: Ikan inggu (*Amphiprion ocellaris*), juga dikenali sebagai percula palsu, atau ikan Inggü dalam Bahasa Melayu ialah spesies hiasan marin yang popular dihargai kerana warna-warna cerah dan daya tahannya. Kajian ini memberi tumpuan kepada pembiakan kurungan dan penghibridan ikan inggu untuk mengurangkan tekanan ke atas populasi liar dan menyokong pemuliharaan. Dari 2018 hingga 2023, ikan hiasan seperti ikan emas, betta dan ikan inggu menyumbang dengan ketara kepada ekonomi eksport Malaysia, dengan pengeluaran 2022 mencecah 200 juta ikan dan hasil RM 453 juta. Disebabkan permintaan yang meningkat, penuaian berlebihan telah menjadi ancaman kepada stok liar. Penyelidikan ini, yang dijalankan di Institut Penyelidikan Perikanan di Batu Maung, menguji kaedah pembiakan menggunakan ikan inggu yang ditangkap liar dan dibeli di kedai di tapak semaian dalaman dan luaran. Penemuan menyerlahkan kepentingan mengurus kepelbagaian genetik dan risiko penyakit. Kajian itu meliputi teknik pembiakan, penghibridan, pertimbangan etika dan impak pemuliharaan—menekankan bahawa amalan pembiakan yang bertanggungjawab, disokong oleh sains dan kerjasama, adalah kunci untuk melindungi biodiversiti sambil mengekalkan industri ikan hiasan.

Introduction

The marine ornamental fish trade is a thriving multimillion-dollar global industry. The global ornamental fish industry is valued in the billions of USD, with key exporters including Indonesia and the Philippines, supplying a variety of marine species to major markets such as the United States, Europe, and Japan. In 2022, Malaysia's ornamental fish export industry was valued at approximately USD 13.4 million, ranking the country as the 8th largest exporter worldwide. Key export destinations include China, Singapore, and the United States (Bothare, 2023).

Amphiprion ocellaris, commonly known as the false percula or ocellaris clownfish is one of the most popular and iconic ornamental fish species. In Malaysia, it is locally called “ikan inggu” due to its vivid orange body and distinct white bands. Clownfish account for an estimated 25 million individuals traded annually worldwide. Belonging to the family Pomacentridae and subfamily Amphiprioninae, these small, brightly coloured fish are easily recognized by their oval, laterally compressed bodies, which grow up to 11 cm in length. Their symbiotic relationship with sea anemones provides protection from predators, enhancing their appeal in the aquarium trade due to their beauty, resilience, and fascinating behaviour. The retail prices of clownfish in the Malaysian market range from \$10 to \$50 USD per fish, influenced by factors such as species and colouration.

Despite their popularity, *A. ocellaris* populations—both globally and in Malaysia—are under threat from overharvesting for the aquarium trade and coral reef degradation. In Malaysia, wild *A. ocellaris* are primarily found in coral-rich coastal waters of Sabah and Sarawak, where the South China Sea provides an ideal environment for various clownfish species. These include *A. ocellaris*, *A. percula* (true percula), and *A. clarkii* (Clark’s anemonefish), all of which form symbiotic relationships with native sea anemones such as *Heteractis magnifica* and *Stichodactyla gigantea*. However, the rising demand for wild-caught specimens often exceeds sustainable harvesting levels, contributing to population declines (Praveena et al., 2012). Without effective conservation strategies and responsible aquaculture development, these declines may persist, threatening marine biodiversity.

Captive breeding of *A. ocellaris* in Malaysia has made strong progress in recent years. Early success by Liew et al. (2006) showed its potential, although challenges in developing broodstock from F1 generations meant wild pairs were still needed. Later studies, such as by Khoo et al. (2018), provided new insights into the species’ growth, diet, and reproductive traits, improving breeding practices. Local interest is growing, shown by more breeders and events like Malaysia’s first clownfish competition. While efforts have focused on nutrition, breeding conditions, and hybridization to reduce pressure on wild populations, important gaps remain—especially on genetics, wild population impacts, and disease control in aquaculture. This article reviews recent progress and challenges in breeding and hybridizing *A. ocellaris*, looks at the ethical and conservation implications, and suggests ways to improve sustainability. It also highlights some of the R&D held at the Aquarium Tunku Abdul Rahman (AkuaTAR), FRI Batu Maung, Penang.

Methodology

To compile scientific references on clownfish breeding, search platforms such as Google Scholar, PubMed, and ScienceDirect were employed using targeted keywords: “captive breeding of clownfish,” “selective breeding of *Amphiprion ocellaris*,” “genetic diversity in clownfish,” and “aquaculture and hybridization of clownfish.” These terms yielded peer-reviewed articles, case studies, and experimental research focusing on aquaculture practices, genetic implications, and sustainability of captive clownfish populations. Complementary insights were drawn from reports by government agencies, including the U.S. Fish and Wildlife Service (USFWS) (USFWS, 2024), and the Food and Agriculture Organization (FAO) (FAO & NACA, 2023), which offered guidance on sustainable aquaculture and ornamental fish breeding frameworks. Additionally, publications from professional bodies such as the World Aquaculture Society and *Aquaculture Magazine* provided industry-specific perspectives on the ecological and commercial dimensions of clownfish aquaculture. Collectively, these sources were instrumental in understanding how captive breeding alleviates pressure on wild populations while supporting species resilience and long-term sustainability.

Breeding and hybridization of clownfish

Captive breeding and hybridization experiments on *Amphiprion ocellaris* were conducted at the Fisheries Research Institute (FRI) in Batu Maung, Pulau Pinang, Malaysia, from December 2019 to December 2020.

Broodstock management

Securing adequate marine ornamental broodstock is essential during the early phases of seed production to maintain genetic diversity within the population (Duncan 2013). In aquaculture hatcheries, productivity and efficiency can be enhanced by effective broodstock management, reproductive control, and genetic improvement. Through selective breeding over multiple generations, desirable traits such as disease resistance and rapid growth can be emphasized, resulting in higher-value and more robust stock. Reproduction control typically involves two key categories: crucial points—which determine the success of breeding efforts, and management points—which focus on the operational practices that support reproductive efficiency.

The initial clownfish stock was acquired in 2019 from Langkawi (Pulau Anak Datai) which is located off the northwest of Langkawi, Malaysia (Figure 1). The perfect parent size for breeding purposes is 5 cm for males and 7 cm for females.



Figure 1. Location of sample collection (Pulau Anak Datai, Langkawi). Source: Google (n.d)

Clownfish health and vigor depend on feeding it a balanced diet. Clownfish are omnivores by nature; their primary sources of food include algae, tiny crustaceans, and zooplankton. It's crucial to try and mimic their natural diet as much as you can in a home aquarium. The impact of feeding young anemonefish insect meal (*Hermetia illucens*) and discovered no adverse impacts on growth, survival, or stress response (Vargas-Abúndez et al., 2019). The rearing of majority of marine fish larvae is done mainly with live feed like rotifer and artemia. The critical factor for their dietary value is their content of n-3 HUFA (Rainuzzo et al., 1997). The Otohime brand and TDO are the two primary commercial food types used in the study of clownfish egg hatching in the aquarium. These food types are fed to the current brood on a daily basis. Another crucial factor is the quantity of original populations that must be domesticated and the process of adapting to artificial environments. Generally, anemone provides protection for these fish. These fish have weak swimming abilities and are more vulnerable to predators in open waters. Clownfish have a unique mucus covering them that protects them from

the host anemone's sting cells. Clownfish can feed on floating planktonic creatures while feeling safe inside the anemone's tentacles (Denver Zoo, n.d). Clownfish and anemones have a symbiotic relationship that has allowed them to adapt to life on the reef. Because clownfish are little, larger fish would likely eat them if the anemone's stinging tentacles didn't shield them from predators.

Fish culture primarily prioritizes high production without taking management into account, it is challenging to avoid infections. As a result, the cultural area is overly congested and improperly managed. Stress and disease epidemics, particularly infectious diseases, are easily caused by this. One of the prevalent viral diseases affecting marine fish cultures, lymphocystis disease (LCD), was identified in 1874 (Zhang et al., 2004). Clownfish with lymphocystis present clinical signs such as white spots and warts typically associated with the disease, and they confirmed the identity of a lymphocystis disease virus (LCDV) in anemonefish tissues (Lam et al., 2020). In the studies conducted at AkuaTAR, we did not use live fish as a breeding place for the fish to live, we used mosaic pieces with the size of 15cm x 15cm and 1.5cm thick arranged in a triangular shape (Figure 2). The rough and flat part of the mosaic is arranged on the inside which is the place where the eggs are attached to the clownfish.



Figure 2. Mosaic Pieces Arranged in a Triangular Shape.

Spawning induction

The nursery breeding procedure involves three main stages: (a) rearing parent fish, (b) culturing zoosperm, and (c) raising juvenile fish. While spawning in captive clownfish is not induced directly within the nursery, various techniques—such as environmental conditioning and hormonal stimulation—are applied elsewhere to trigger reproduction. In the wild, clownfish typically lay their eggs on smooth rock surfaces near their host anemones, after which the male fertilizes them externally. According to our study, this reproductive mode is classified as external fertilization. Depending on age and maturity, female clownfish may lay between 100 and 1,500 eggs per spawning cycle.

In the wild, clownfish eggs usually hatch around the full moon, possibly to take advantage of high tides that help carry hatchlings away from predators. Hatching typically occurs within 6 to 10

days, depending on sea temperature. In controlled environments, research shows clownfish eggs take about 7 to 8 days to hatch—slightly longer during rainy, cooler weather. Reproduction in anemonefish can be affected by artificial lighting at night. At AkuaTAR, no artificial light is used; instead, natural sunlight shines through a translucent roof to illuminate the hatchery tanks.

A study by Fobert et al. (2019) found that exposing clownfish to artificial light at night can reduce their hatching and reproduction rates. Larvae raised under different lighting and tank conditions showed that both light and tank color greatly affect their survival and development. Using fiberglass tanks helps limit light exposure, but covering the top of the tank is still important to prevent disturbances. Although this reduces light even more, it creates a stable environment that supports healthy growth. Since clownfish larvae are visual feeders, they need the right background and lighting to help them locate food effectively (Ignatius et al., 2001).

At AquaTAR, our study observed that male clownfish take an active and bold role in egg care, supporting findings by Dhaneesh et al. (2009), who highlighted the male’s central role in egg protection. After each hatching cycle, the same broodstock was able to spawn up to seven to ten additional times (Figure 3). Successful spawning was linked to several key factors: healthy broodstock, proper tank design, compatible pair selection, stimulation of breeding behavior, consistent monitoring, attentive care of eggs and larvae, and thorough documentation. Together, these practices are vital to ensuring the long-term success of artificial reproduction and the conservation of clownfish populations.

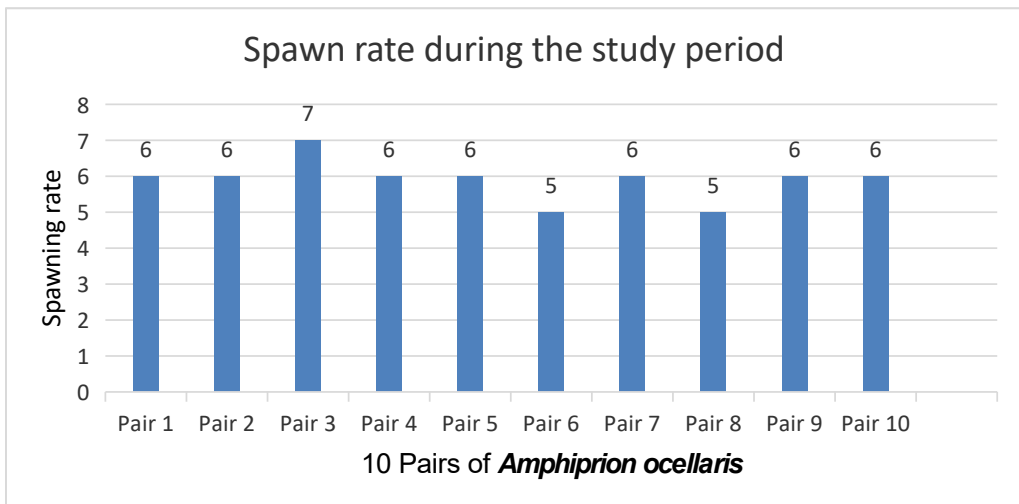


Figure 3. Spawn rate during the study period (each pair is referring to one male and one female).

Thus, nutrition and environmental conditions play a role in this scenario. A study found that, spawning on *Amphiprion* can happen up to three times per month (Balamurugan et al., 2013). The study found that clownfish’s productivity in broodstocks can be increased by random spawning and interbreed crossing. The number of effective breeding numbers (N_e) can be raised by using this technique. To achieve the study’s objectives, the parental specimen is housed in a tank with a triangular mosaic, as mentioned earlier. This arrangement enhances the environment, addresses territorial and behavioural factors, simulates natural habitats, and promotes reproductive behaviour. These factors collectively contribute to the well-being of the fish and increase the likelihood of successful breeding outcomes for the study.

During the duration of the study, the parental specimen was housed within a tank measuring 60cm x 40cm x 40cm (Figure 4), alongside a triangular mosaic. The chosen parental specimen was paired according to their respective sizes and maintained in pairs until they reached the stage of egg-laying. The number of brood stocks mating, the sex ratio, the mating techniques, and the changes in family size all contribute to a hatchery's N_e value (Warwick et al., 1995; Falconer and Mackay, 1996). In the event that the productivity of the broodstock declines, pedigreed mating may be conducted. Because there is an increase in N_e without an increase in the quantity of brood stock populations, this strategy will be able to raise the productivity of broodstock fish.



Figure 4. Tank for the parental specimen.

Hybridization

Hybridization is used in captive breeding to increase genetic diversity and improve traits, such as growth or disease resistance (Zecherle et al., 2021). In agriculture, it helps produce stronger crops and animals. In technology, it means combining systems to create better-performing products (Liu et al., 2022). Hybridization also supports adaptation and can lead to new, more resilient species (Schley et al., 2021). It encourages innovation by blending ideas across fields, improves efficiency, and enhances how resources are used. In business, hybrid models mix traditional and modern practices for better performance (Zecherle et al., 2021). Benefits include stronger, more adaptable organisms, flexible technologies, and creative breakthroughs (Hahn & Ince, 2016; Goswami et al., 2023). Cultural hybridization can lead to new art forms and diverse cultures (Tuncer, 2023). However, there are downsides: it may harm genetic purity, cause invasive species, create compatibility issues in tech, or face resistance from traditionalists in both culture and business (Tuncer, 2023).

There are still several challenges to overcome. Technological hybrid systems can be complex, making integration and maintenance more difficult. These systems often require extra resources and specialized knowledge, adding to their overall complexity (Wang et al., 2023). Clownfish, known for their bright colours and unique behaviours, have also benefited from selective breeding in aquaculture. This has led to new colour variations that appeal to aquarium enthusiasts (Pratiwi et al., 2022). Genetics can help enhance and stabilize these traits. Other key areas of interest include their social behaviour, adaptability, and resilience to environmental changes.

The primary objective of the breeding in the study is to cultivate clownfish kinds that exhibit adaptability to captivity, hence demonstrating behaviors that are beneficial to flourishing in artificial habitats. The use of technology in the creation and maintenance of settings that replicate natural circumstances is significant. This is achieved through the utilization of improved lighting, automated feeding systems, and artificial structures that mimic the characteristics of a reef ecosystem, which involves many proven technologies (Levy et al., 2022).

To summarize, although hybridization presents multiple benefits in different fields, it is essential to thoroughly evaluate possible disadvantages and ethical consequences to guarantee responsible and sustainable adoption. Essentially, the development of improved characteristics in clownfish, whether in their native environments or in captivity, is influenced by a blend of biological elements, deliberate breeding, and technological interventions. The production of clownfish types with desirable qualities, which are well-suited for aquarium hobbyists while ensuring their well-being and adaptability in artificial habitats, is influenced by both biology and technology.

From the study, placing excessive focus on characteristics without taking into account the total genetic well-being may result in unforeseen outcomes, underscoring the significance of a well-rounded breeding approach. Strategies for the preservation of genetic health including managing the founder population. The incorporation of novel individuals or genetic material derived from wild populations on a regular basis can effectively contribute to the preservation of genetic diversity within captive breeding programs. In the study, all the breeding activities genetic composition were recorded. Maintaining comprehensive documentation of the genetic composition of the pioneer population facilitates the monitoring of genetic relatedness and the effective management of genetic diversity. On the other hand, to prevent the detrimental implications of excessive inbreeding, it is important to strike a balance between selecting for specific qualities and conserving genetic diversity and ensuring a breeding population of adequate size mitigates the likelihood of inbreeding depression (Moss & Moss, 2009).

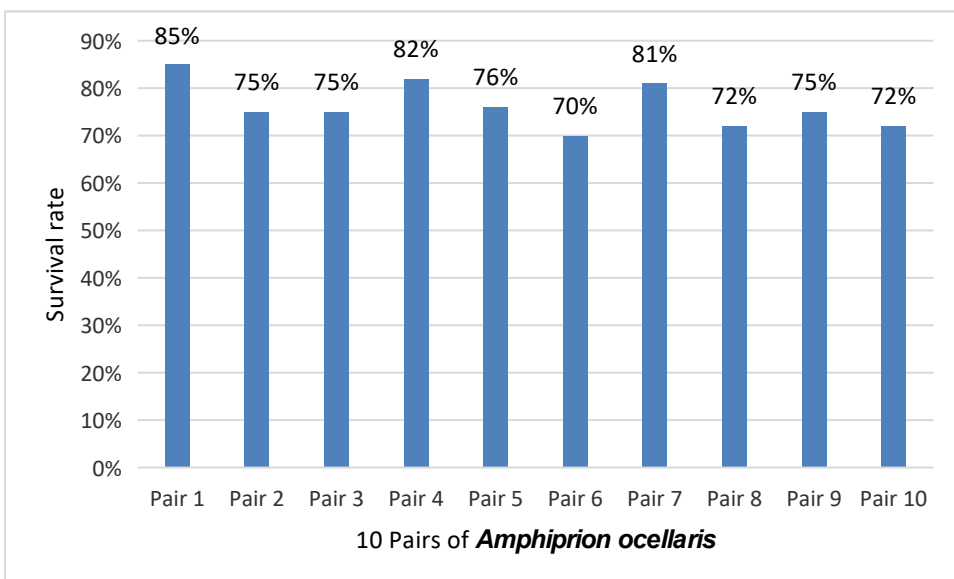


Figure 5. The survival rate of clownfish larvae in 100 days (each pair is referring to one male and one female).

In conclusion, the preservation of genetic variety and the mitigation of inbreeding depression are pivotal factors to be taken into account in the context of captive breeding of clownfish in captive breeding. The preservation of genetic health and long-term sustainability of captive clownfish populations necessitates the implementation of strategic hybridization, meticulous selection, and continuous genetic monitoring. The implementation of responsible breeding methods is crucial for the sustained efficacy of conservation endeavours and the overall welfare of these distinct marine species that are now held captive.

The group of researchers involved consists of researchers with a variety of skill backgrounds, in terms of live food preparation, fish farming in tanks, aquarium systems and fish care starting from the recruitment of broodstock from the original habitat. At this point, no specific training is given to the team involved, most of them learn through reading, YouTube and various other sources. Exchanging internal staff to gain knowledge is the normal practices done in order to increase the knowledge among the researchers. Educational programmes play a crucial role in promoting responsible breeding methods by imparting knowledge to breeders and the general public regarding the significance of genetic variety (Burens, 2025).

The research findings indicate that the assessment of clownfish survival rates (Figure 5), as well as fish birth rates, does not rely on the quantity of original eggs. Instead, it is determined by the number of eggs that successfully hatch on the initial day of hatching. To determine the survival rate of the fish larvae, data is collected over a period of 100 days. The rationale behind refraining from recording the overall egg count is attributed to the presence of multiple elements that can impede the hatching process, including but not limited to noise interference, feeding errors, dirtiness, and the demands of hatching activities. This implies that if the first spawning yields 300 eggs that successfully reach the hatching stage, it is probable that only half of the initial egg count will be retained.

Captive breeding techniques

Captive breeding involves the controlled reproduction and rearing of fish outside their natural habitats—such as in hatcheries or aquaculture facilities (Fraser, 2008). It serves various purposes, including commercial production, conservation, and stock enhancement. This approach helps reduce pressure on wild fish populations while supporting sustainability efforts. However, breeding animals in captivity requires strong ethical practices and a deep understanding of species needs. Robert (2009) emphasized that captive environments can cause genetic and phenotypic changes affecting reintroduction success, highlighting that effective captive breeding must provide suitable rearing conditions to ensure offspring survival and adaptation after release. At AkuaTAR, creating optimal conditions for clownfish breeding involves careful tank setup and monitoring. Selecting compatible pairs and closely observing their behavior is key to the success of the program. During the study, a white band—a notable feature—began appearing on clownfish larvae by the 15th day of growth.



Figure 6. Fish Selection Stage for Breeding

In the 1970s, marine biologist Martin Moe became the first to successfully breed *A. ocellaris* in captivity, showing that they could be raised without relying on wild stocks (Moe, 2012). His breakthrough sparked global interest in breeding methods and larval rearing, leading to the rise of commercial clownfish hatcheries. Today, captive breeding programs continue to grow, incorporating hybridization, selective breeding, and sustainable practices to protect wild populations.

Understanding clownfish reproduction is key to successful captive breeding. Clownfish are *protandrous hermaphrodites*—they begin life as males and may later become females (Fricke & Fricke, 1977). In a single sea anemone, a dominant female, a male, and several juveniles may live together. If the female dies, the male becomes female, and a juvenile matures into the new male (Thresher, 1984). *Amphiprion ocellaris* can breed year-round in tropical seas (Figure 6), with spawning often aligned with the full moon, which may aid larval dispersal. The female lays orange eggs on a clean surface near the nest, and the male fertilizes them by passing over them. The eggs are attached by a fine thread and take about 6–8 days to hatch, depending on water temperature.

During hatching, water changes are avoided for 15 days to prevent temperature stress. From day six, dirt siphoning begins, continuing until the juvenile fish are transferred around day 25. Clownfish larvae go through a planktonic phase between days 8–12 before settling. Males lead egg care, fanning them and removing infertile or infected ones (Hadie et al., 2023). Once hatched, larvae are independent of their parents.

Larval rearing

When reared in indoor tanks, clownfish larvae begin to transform into fry around 20 days after hatching. Their eggs usually take 6 to 10 days to incubate. After hatching, the larvae require close care and proper feeding for about 10 to 15 days to successfully reach the juvenile stage in their designated tank (Figure 7).



Figure 7. Larval Tank.

The clownfish larvae exhibited distinct fish characteristics, as evidenced by the emergence of a white band on the 15th day of growth, as observed during the period of the study. The clownfish *A. ocellaris*' life cycle is thoroughly described in the work, while the larval rearing protocol is described well (Roux et al., 2019). The life cycle of clownfish was drawn as follow (Figure.8).

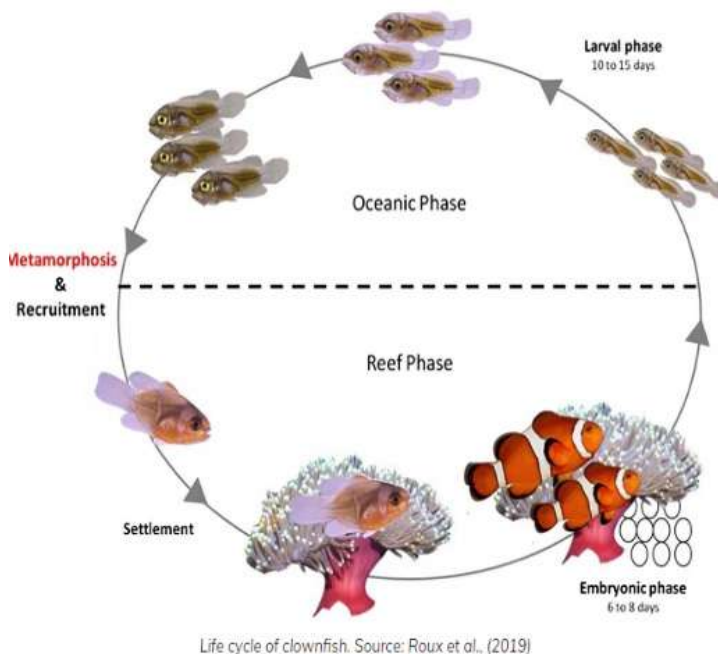


Figure 8. Life cycle of clownfish. Source: Roux et al., (2019).

Challenges and ethical considerations

Disease Management

The captive breeding programs are greatly endangered by disease outbreaks. Including the challenge lies in the fact that breeding facilities frequently function within enclosed systems characterized by a high population density, so establishing a favorable setting for the swift dissemination of diseases. However, overcrowded environments might enhance the spread of diseases, posing difficulties in preventive (Huntingford et al., 2006). In the study carried out, the clownfish that were cared for also suffered from disease infections like other ornamental fish, all caused by water quality management. In general, the diseases that often affect purple fish are white spot and Brooklyn disease. The researchers will regulate the feeding process, dispose of surplus food, prevent excessive food from spoiling in the tank, introduce a UV lamp into the water storage tank before to usage, and perform regular water changes in case the water quality declines.

The presence of limited immunity heightens vulnerability to illnesses, hence requiring the implementation of proactive preventive measures (Semple & Dixon, 2020). The immune systems of clownfish can be compromised by many stressors, including alterations in water quality, handling, or transportation. The susceptibility of humans to infections is heightened by stress, underscoring the significance of stress management in the realm of disease prevention. Which it is suggested to do the study in future time. Researchers are also ready with the challenges in disease detection, such as inconspicuous manifestations including the detection of clownfish diseases might be problematic due to the presence of modest or nonspecific symptoms. One potential risk associated with the delayed discovery of a disease epidemic is the potential for widespread infection and heightened challenges in containment efforts (Cheng et al., 2022).

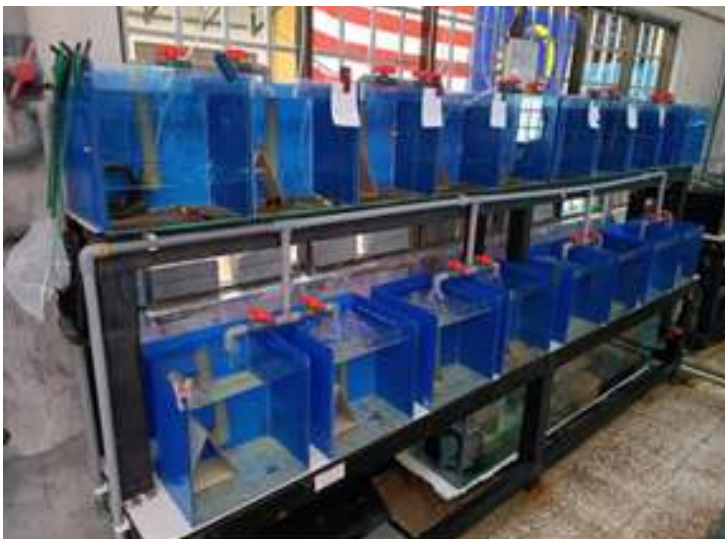


Figure 9. Quarantine tank.

Some clownfish can carry diseases without showing symptoms, acting as silent carriers that spread infections. This makes it harder to control outbreaks, especially since diagnostic tools to detect specific clownfish diseases are often limited (Rao et al., 2013). In small, enclosed systems like hatcheries, treatment options are also restricted. Medications and quarantine procedures can cause stress, sometimes worsening fish health (Lieke et al., 2020). Over time, pathogens may even become resistant to common treatments, requiring new approaches (Wainberg et al., 2017).

To reduce these risks, strict quarantine protocols are used for new arrivals, and water quality is carefully maintained to lower stress and improve fish health (Santikawati et al., 2023) (Figure 9). Routine practices include changing 20% of the water weekly, 70% monthly, daily siphoning of waste, and topping up water as needed. Selecting breeding pairs with natural disease resistance was part of this study's strategy. Staff were also trained in disease prevention, detection, and treatment (Varela et al., 2022). Overall, effective disease management in clownfish breeding requires a well-rounded approach, combining good hygiene, early detection, proper treatment, and strong collaboration within the aquaculture community.

Ethical concern

Ethical considerations play a key role in the captive breeding and hybridization of clownfish. This includes concerns about animal welfare, genetic health, and the impact on wild populations. Ensuring good quality of life for captive clownfish means providing appropriate tank sizes, environmental enrichment, and adequate nutrition (Boumis, n.d). Striking a balance between attractive traits and the fish's well-being is essential. It's important that clownfish in captivity can still express natural behaviors—like social interaction and forming bonds with anemones (Émie et al., 2021). Enrichment activities help stimulate their natural environment, improving their mental and physical health. However, selectively breeding for specific colors or features can risk reducing genetic diversity and causing inbreeding issues (Van Wyk et al., 2009). Responsible hybridization must consider long-term effects, such as infertility or genetic weaknesses (Marcionetti et al., 2019).

Ethically, captive breeding should not encourage unsustainable collection of wild clownfish. Breeders should use responsible sourcing to avoid harming declining wild populations. Releasing captive-bred or hybrid clownfish into the wild could lead to ecological disruptions if they are not native, potentially creating invasive populations (Semmens et al., 2004).

Transparency in breeding methods is vital. Sharing knowledge about ethical practices and raising public awareness helps ensure accountability and informed choices (Biasseti et al., 2020). Collaboration between breeders, researchers, and conservation groups can help protect wild clownfish and their habitats (Palmtag, 2017). In summary, ethical breeding of clownfish calls for responsible practices that prioritize animal welfare, safeguard genetic integrity, avoid negative impacts on wild stocks, and support education and sustainability (Moorhead & Zeng, 2010).

Release and reintroduction

Captive breeding plays an important role in conserving clownfish by reducing the pressure on wild populations. It provides a sustainable alternative to wild-caught fish for the aquarium trade, helping protect natural habitats (Rhyne et al., 2017). Some programs have successfully bred and raised clownfish in captivity, offering potential for reintroducing them into the wild. This ensures that aquarium demand can be met without further harming wild stocks and gives local breeders and hobbyists the opportunity to raise their own fish. Captive breeding also helps educate the public about clownfish conservation and the environmental impact of the aquarium industry. When done responsibly, it helps maintain genetic diversity and prevents inbreeding. However, challenges remain. Clownfish have complex life cycles, especially during larval stages, which require expertise and resources to manage (Önal et al., 2008). Issues such as limited staff, disease outbreaks in crowded facilities, and the risk of reduced genetic health in large-scale production systems still need to be addressed (Olivotto et al., 2011).

There are also risks in releasing captive-bred or hybrid fish into the wild, as they might disrupt local ecosystems. Still, with proper controls, captive breeding is a valuable tool to prevent extinction caused by poor handling of wild populations. Success depends on collaboration between researchers, breeders, and conservationists. Selective breeding programs should focus on preserving genetic health (Allendorf et al., 2022), while education and certification programs can promote sustainable practices and ethical purchasing. Protecting natural habitats alongside captive efforts is also crucial (Attard et al., 2016). In summary, while captive breeding supports clownfish conservation, ongoing work is needed to improve breeding practices, address risks, and ensure these efforts align with broader environmental goals.

Release and Reintroduction

This article explores both the practical and ethical aspects of releasing captive-bred clownfish into the wild, focusing on habitat suitability and ecological impacts. Before any release, it's crucial to assess whether the chosen site has the right water quality, temperature, and host anemones. Releasing clownfish into unsuitable or unfamiliar environments may be harmful, and predators in the area must be considered. In some cases, training captive fish to recognize threats can improve survival chances (Douglam, 2009; McCleery et al., 2014). There are concerns that introducing non-native or hybridized clownfish could disrupt local ecosystems or compromise the genetic integrity of wild populations (O'Sullivan et al., 2020). Genetic monitoring can help track interbreeding and assess long-term impacts. However, when carefully planned, reintroductions can support threatened populations—especially where overharvesting or habitat loss has occurred (Aziz, 2014).

Successful reintroduction depends on how well the captive fish adjust to the wild, including finding food, avoiding predators, and competing for space (Griffin et al., 2000). These programs can also be costly, requiring long-term funding and consistent monitoring. Pre-release studies are essential for understanding the species' ecological needs and preparing them for life in the wild (Ardoin et al., 2020).

Key steps include:

- Conducting ecological impact assessments
- Monitoring genetics post-release (Pärli et al., 2021)
- Gradually acclimating fish to natural conditions
- Running small-scale pilot releases (Bell et al., 2006)
- Raising public awareness and involving local communities

In conclusion, releasing captive-bred clownfish can aid conservation if done responsibly. This requires careful planning, ethical consideration, and collaboration across researchers, breeders, and communities to ensure long-term success and minimal disruption to wild ecosystems.

Conclusions

This article highlights the progress and challenges in the captive breeding and hybridization of *A. ocellaris*, focusing on genetic diversity, conservation, disease control, and ethical considerations. Captive breeding has proven essential for reducing pressure on wild populations and sustaining the ornamental fish industry. However, maintaining genetic health requires careful management to avoid inbreeding. Hybridization can improve resilience and introduce new traits, but must be applied responsibly.

Effective disease management—through biosecurity, health monitoring, and selective breeding is crucial for maintaining healthy captive populations. Ethical concerns such as welfare, stress reduction, and habitat enrichment are equally important. Any reintroduction of captive-bred fish into the wild should be based on sound ecological and genetic assessments. The study recommends ongoing research into genetic monitoring, behavioral needs, and stress reduction, alongside stronger collaboration among breeders, researchers, and government bodies. Together, these efforts can help establish sustainable, science-based guidelines for the industry.

In summary, when managed responsibly, the captive breeding and hybridization of *A. ocellaris* offer clear conservation and economic benefits—helping to protect this iconic species both in aquariums and in its natural habitat.

Acknowledgments

The authors wish to thank Dr. Azhar bin Hamzah, the Senior Director of the Institute of Fisheries Research, Department of Fisheries, for making this project possible. The staff of AkuaTAR who have contributed their work is also appreciated. Our genuine appreciation is likewise stretched out to all research colleagues, field support individuals, programmers and data processing members for their committed exertion and responsibility in this study.

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INSTRUCTION TO AUTHORS

MALAYSIAN FISHERIES JOURNAL

Aim and Scope

The journal seeks to provide a forum for dissemination of research findings in all aspects of fisheries science. Manuscripts describing research work relevant to local communities are most welcome to aid in the advancement of sustainable fisheries. The standardized format set below is an adaption from some international journal.

Submission of Papers

A paper is considered for publication on the understanding that:

- It reports original unpublished work
- It is approved by all named authors
- It does not contain tables and figures that have been published elsewhere
- All acceptable manuscripts will be reviewed by the Publication Committee
- Acknowledgement and action on each point raised by the reviewer will be requested from the author if the manuscripts to be accepted

Different type of Submissions

1. Full length paper

These should describe new and carefully confirmed findings and experimental procedures that should be given in sufficient detail for others to verify work. The length of a full paper should be the minimum required to describe and interpret the work clearly. The paper should comprise the following sections: (a) Abstract; (b) Introduction; (c) Materials and Methods; (d) Results; (e) Discussion; (f) Acknowledgement; (g) References; (h) Tables; (i) Legends to figures; (j) Figures. The results and discussion section may be combined.

2. Short Communication

A Short Communication is suitable for recording the results of complete small investigation or giving details of new methods, techniques or apparatus, not more than 3000 words. The style of main sections need not conform to that of full-length papers. Progress reports are not acceptable.

3. Short Notes

Short Notes are one to two printed pages in length. They are suitable for reports of simple findings such as properties of an already well-described enzyme or of observations not requiring elaboration. They should be written with a short summary, with no main sub-division, may contain one table or figure, or two if the text is brief and no more than three references.

4. Technical Communication

These are reports of processes or procedures which may be published as an annex to a full length of paper or on their own provided that the work is of sufficient interests to other workers in the field.

5. Review Papers

These should be centered on current issues that are of interests to all. The length of the paper is between 6000 – 10000 words. The references must be more than 30.

Preparation of Manuscripts

Manuscript should be prepared in Microsoft Word. The paper must be typed with a double spacing throughout, including references, tables, footnotes, figures legends, etc on A4 size paper leaving margins of 25 mm minimum. Line numbers should be insert for review purposes. Headings should be centered, upper case in bold, size 14. Sub-headings should be lower case, centered and in **bold**. Sub-sub-headings should be in *italics* at left margin. The font used throughout your document should be in Times New Roman, 12-point font size.

Title Page:

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Abstract and Keywords

Abstract: Hanging indent, *italic*, **bold**, follow by text

Two abstracts required: English and Bahasa Malaysia version

Provide: i. No more than 200 words summarizing the main points of the paper

- ii. Up to six keywords or phrases

Introduction

It should include key references to appropriate works and up-to-date primary literature. The rationale of the research undertaken should be explained. The introduction should clearly state the aims and objectives of the paper.

Materials and Methods

Materials and Methods should be described in sufficient detail to allow the work to be repeated. Specify and describe the study site and test animals where appropriate. Sub headings are used to itemize the main parts. Materials and methods should be written in the past tense either in active or passive voice. In this section, study dates, number of subjects, groups, evaluation criteria, exclusion criteria and statistical methods should be described sequentially. The origin of materials and/or suppliers of equipment should be named if necessary.

Results and Discussion

The sections may be separated, though authors may find it's easier to combine them. Use tables or graphs as appropriate but do not repeat information in the text. The reproducibility of the findings must be clearly stated, the number of times the experiment was conducted, the number of replicate samples, etc., should be stated. Statistical analysis of results must specify the procedure being used with a reference given. If results are given as a percentage of a control value, the 100% should be given.

Discussion should provide the explanation and interpretation of results or findings by comparing with the prior studies. It should bring out those essential points of the work, the implications and practical significance of the findings, their limitation and relevance to previous studies. It should not be a recapitulation of the results.

References

References in text should be cited as: Smith (1993) or (Smith, 1993). Two authors as: Smith and Brown (1993) or (Smith and Brown, 1993). Three or more authors as: Smith et al. (1993) or (Smith et al., 1993). A series of references should be appearing in chronological order, e.g., White and Black 1991; Black and White 1992. References to papers by the same authors in the same year are distinguished by letter a, b, etc. (e.g., 1989a, or 1991a, b). Publications having no obvious authors are cited as Anon. (1990) in the text and bibliography. References with 4 and more authors should be written as: Abbasi, A. A., Paparidis, Z., Malik, S., Goode, D. K., et al. (2007). References to grey literature such as in-house reports, contract reports and non-referred papers are not appropriate and should be avoided. At the end of papers, References are listed in alphabetical order by the first word in the reference (usually the author's last name). References with three or more authors should be placed in chronological order after considering of the names of the first and second authors. The author must ensure that references cited in the text agree with those listed in the bibliography. Some sample reference styles follow:

a) Citation for Journals

i. In print

Sequence of citation: author's name, initials (for each author)(year of publication). Full title of paper. *Name of journal* (abbreviated in accordance with the Bibliographic Guide), **volume** (issue nu), first page – last page.

Example: Saha, B. C. and/& Zeikus, J. G. (1989). Improve method for preparing high maltose conversion syrup. *Biotechnology and Bioengineering*, **34**, 229-303.

Example: Debnath, P. P., Delamare-Deboutteville, J., Jansen, M. D., Phiwsaiya, K., et.al (2020). Two-year surveillance of tilapia lake virus (TiLV) reveals its wide circulation in tilapia farms and hatcheries from multiple districts of Bangladesh. *J Fish Dis.* **43**(11),1381-1389.

ii. Online

These references are formatted the same way as the print versions, except the DOI or URL is included at the end. If the article has a corresponding DOI number, use it instead of the URL. No URL? Use the homepage of the journal's website for the URL.

Author's Last name, F. M. (Year published). Title of article. *Title of Journal*, volume number (issue number), page range. <https://doi.org/10.xxxx/xxxxxx> OR URL

Example:

Spreer, P., and/& Rauschnabel, P. A. (2016). Selling with technology: Understanding the resistance to mobile sales assistant use in retailing. *Journal of Personal Selling and Sales Management*, **36**(3), 240-263. <https://doi.org/10.1080/08853134.2016.1208100>

b) Citation for Books

i. Sequence of citation for print books: author's or editor's name, initial,(year of publication), *book title*. Publisher, and place of publication.

Capitalize the first letter of the first word of the title and any subtitles, as well as the first letter of any proper nouns. The full title of the book, including any subtitles, should be stated and italicized.

Example: Primrose, G. B. (1987). *Modern Biotechnology*. Blackwell Scientific, Oxford.

ii. Citations for Edited Books

Most edited books state on the cover or title page that they are edited by an author or multiple authors. The format is the same as a print book, except the editor's name is in the author's position. Include a parenthesis afterwards with the abbreviation (Ed.) for an edited book by one author or (Eds.) for an edited book with two or more authors.

Editor, F. M. (Ed.). (Year published). *Title of edited book*. Publisher.

Example:

a) Primrose, G.B. (Ed.). (1987). *Modern Biotechnology*. Blackwell Scientific, Oxford.

b) Gudding, R., Lillehaug, A. and Evensen, O. (Eds.). (2014). *Fish Vaccination*, John Wiley & Sons Ltd., UK.

iii. Citations for Chapters in Edited Books

Some edited books contain chapters written by various authors. Use the format below to cite an author's individual chapter in an edited book.

Chapter author's Last name, F. M. (Year published). Title of chapter. In F. M. Last name of Editor (Ed.), Title of book (p. x or pp. x-x). Publisher.

The title of the chapter is not italicized, while the title of the book is. The chapter author's name is reversed at the beginning of the reference, but the editor's name is written in standard order.

Example:

a) Longacre, W. A., and Ayres, J. E. (1968). Archeological lessons from an Apache wikiup. In S. R. Binford and L. R. Binford (Eds.), *Archeology in cultural systems* (pp. 151-160). Blackwell, Oxford, UK.

In the above example, Longacre and Ayers are the authors of the individual chapter and Binford and Binford are the editors of the entire book.

b) Gudding, R. (2014). Vaccination as a preventive measure. In R. Gudding, , A. Lillehaug, and O. Evensen, (Eds.), *Fish Vaccination* (pp. 12-21). John Wiley & Sons Ltd, West Sussex, UK.

c) Citations for conference/proceedings

Conference proceedings published as a whole book follow the same reference format as whole: i) journal, ii) edited books or iii) book chapter

Example:

i. Duckworth, A. L., Quirk, A., Gallop, R., Hoyle, R. H., Kelly, D. R. (2019). Cognitive and noncognitive predictors of success. *Proceedings of the National Academy of Sciences, USA*, 116(47), 23499-23504. <https://doi.org/10.1073/pnas.1910510116>

ii. Kushilevitz, E., and Malkin, T. (Eds.). (2016). *Lecture notes in computer science: Vol. 9562. Theory of cryptography*. Springer. <https://doi.org/10.1007/978-3-662-49096-9>

iii. Benedel, A. L., Jourdan, L. and Biernacki, C. (2019). Probability estimation by an

adapted genetic algorithm in web insurance. In R. Battiti, M. Brunato, I. Kotsireas & P. Pardalos (Eds.), *Lecture notes in computer science: Vol. 11353. Learning and intelligent optimization* (pp. 225-240), Springer. https://doi.org/10.1007/978-3-030-05348-2_21

a) Citations for Newspapers found Online

Use this structure when referencing a newspaper article found on a website or database:

Author's Last name, F. M. (Year, Month Day of Publication). Title of article. Title of Newspaper. URL of newspaper's homepage

Example:

Rosenberg, G. (1997, March 31). Electronic discovery proves an effective legal weapon. *The New York Times*. <http://www.nytimes.com>

b) Composite works of serials:

Sequence of citation: author's name, initials, year of publication, publisher, place of publication, first and last page no.

Example:

Guilbot, A. and Marcier, C. 1985. Starch. In: Aspinall, G.O. (ed). *The polysaccharides*, Academic Press, New York, pp. 209-283.

c) Full publication details must be given for any citation that does not fit into any of the above categories such as unpublished in-house reports, contract reports, etc.

Acknowledgement

Brief of appreciation to whom it is due.

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Plain Tables should be used for data which cannot be described in the text. Type each table double spaced and position in the manuscript. Table number and caption should be positioned at the top. Explanatory footnotes in lower case letters should be concise to enable them to stand independent of the main text. Tables are numbered with Arabic numerals.

Figures

Figures should be selected to illustrate points which cannot be easily made in the text. They are numbered with Arabic numerals. Graphs, photos and diagrams with caption should be positioned in the manuscript. Diagrams must be drawn and lettered in black ink on good quality white paper for camera-ready use. Lettering should be parallel to the axes. Photocopies, hand-drawn diagrams and typewritten labels are not acceptable. Scale marks on graphs should be within the axes. Graphs should avoid as far as possible large areas of unused space.

Photographs should be well-contrasted black and white prints. For photomicrographs, the magnification should be given a scale (or marker) bar on each photograph and the length of this represents given in the legend. Photographs of the original material should be submitted for the reviewer's scrutiny and the purpose of printing.

Units, Abbreviations and Nomenclature

Use only recommended SI Units. Use superscripts presentation (mg mL⁻¹). Below are few examples of abbreviations of the most commonly used SI units:

Base quantity	Name	Abbreviation
Length	Meter	m
Mass	Kilogram	kg
Time	Second	s
Time	Minute	min
Electric current	Ampere	A
Area	square meter	m ²
Volume	cubic meter	m ³
Frequency	Hertz	Hz

The correct Latin names of organisms must be used on first mention in the text. A widely recognized and designated common name should be used for subsequent mention.

Nomination of Reviewers

To facilitate the review process, authors are kindly requested to recommend two potential reviewers with relevant expertise. Please provide each reviewer's full name, institutional affiliation, and email address.

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FULL NAME ALL AUTHORS: AUTHOR^{1,2}, AUTHOR³, AUTHOR³, AUTHOR¹, AUTHOR⁴,*

¹*Institution with complete current address, including post code*

²*Institution with complete current address, including post code*

³*Institution with complete current address, including post code*

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Abstract: A concise and factual abstract is required and must be written in English (maximum length of 250 words). The abstract should state briefly the purpose of the research, the methods used, the principal results and major conclusions. Please try to keep each sentence as specific as possible and avoid such general statements as ‘The fisheries status are discussed’. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, they must be cited in full, without reference to the reference list. Any abbreviations should be avoided, but if needed they must be defined at their mentioned in the abstract.

Keywords: Provide a maximum of 6 keywords, using British spelling and avoiding general and plural terms and multiple concepts (avoid, for example, ‘and’, ‘of’). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abstrak: Abstrak di dalam Bahasa Malaysia mestilah mempunyai makna dan terjemahan maksud yang sama seperti dalam abstrak Bahasa Inggeris. Penggunaan ‘google translate’ dibenarkan dengan syarat penulis memeriksa kembali setiap patah perkataan dan membuat pembetulan mengikut tatabahasa yang betul.

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Materials

Provide sufficient detail to allow the work to be reproduced. Methods already published

should be indicated by a reference: only relevant modifications should be described. The correct Latin names of organisms must be used on first mention in the text. A widely recognized and designated common name should be used for subsequent mention.

Methods

Sub headings are used to itemize the main parts. Materials and methods should be written in the past tense either in active or passive voice. In this section, study dates, number of subjects, groups, evaluation criteria, exclusion criteria and statistical methods should be described sequentially. The origin of materials and/or suppliers of equipment should be named if necessary.

Sub-section

Third level section should be italic. We do not encourage additional sub-levels after the third level. Please try to make your paper concise and clear.

Units, abbreviations and nomenclature

Use only recommended SI Units. Use superscripts presentation (e.g: mg mL⁻¹) and common abbreviations such as 'm' for meter, 'kg' for kilogram, 'min' for minute and so on.

Results and Discussion

Results should be clear and concise. The discussion should explore the significance of the results of the work. Avoid extensive citations and discussion of published literature. If appropriate, Results can be written in a separate section from Discussion. This especially if the Discussion is extensive and includes all the Results of the study.

Table

Please submit tables as **editable text** and not as images. Tables use double space and 12pt Times New Roman fonts.

Table 1. Use Times New Roman 12 font

Component	Content (% , w/w)
Protien	44.9 ± 0.37
Carbohydrate	22.3 ± 0.94
Water content	13.7 ± 0.02
Ash	6.1 ± 0.19

Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules.

Figures

Figure should be on the left margin. **Embed the figures in the text** with minimum resolution of 300 dpi. Separate **figure files in JPEG or PNG formats should be supplied together with the article**. Ensure that each figure has a caption. A caption should comprise a brief title (not on the figure itself) and a description of the figure. Keep text in the figure themselves to a minimum but explain all symbols and abbreviations used.



Figure 1. Left: Trap one funnel (1F)

Graphs

Graphs must be supplied in figure formats. The fonts of the graph must be clear and readable. Black and white graphs are preferred.

Citation

Please ensure that **every reference cited in the text is also present** in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list, they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Text

All citations in the text should refer to:

- Single author: the author's name (without initials, unless there is ambiguity) and the year of publication (Ratledge, 2002); OR Ratledge (2002).
- Two authors: both authors' names and the year of publication (Triyaswati & Ilmi, 2020);
- Three or more authors: first author's name followed by 'et al,' and the year of publication (Papanikolaou et al., 2011).

Reference list

References should be arranged first alphabetically and then further sorted chronologically. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of Results and Discussion section.

Author contribution (Optional)

Please list the contribution of each author here, e.g.: M.I. designed the research and supervised all the process, L.A. collected and analyzed the data and wrote the manuscript.

Acknowledgments

List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proofreading the article, etc.).

Conflict of Interest (Optional)

Please state any conflict of interest regarding the research or the research funding.

References

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ISSN 1511 -7286



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