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Identifying Populations of the Marble Goby, *Oxyeleotris marmorata* (Bleeker, 1852) by Otolith Shape Analysis and Their Spawning Seasons in Likas Bay, Sabah, Malaysia

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Abstract

The marble goby, *Oxyeleotris marmorata* is native to fresh and brackish waters of Southeast Asia. The yield of wild *O. marmorata* has been decreased drastically and urgent conservation actions are required to prevent extinction. In the Likas Bay, *Oxyeleotris marmorata* is caught throughout the year by local fishermen using various fishing gears. In fisheries management, population is a fundamental biological unit and population identification of a species is important. The population structure of this marine stock was determined by otolith shape analysis and their spawning season in the Likas Bay was determined by ovarian observations. A total of 147 specimens were procured between 2018 and 2019; size ranged from 443 g to 3,264 g BW. The curvature index of the otoliths was analyzed by Bhattacharya's method and found that the frequency distribution of curvature indices is a mixture of two normal components, indicating two different populations. The specimens with mature or spent ovaries were found in samples collected in January – February and September, showing two spawning seasons. The age of each specimen was estimated by counting the vertebral annuli. The estimated age ranged from 5 to 14 years. The minimum size and age of spawners was 338 mm TL, 499 g BW and 5 years old. The findings obtained in this study provide basic biological parameters of *O. marmorata* populations in Likas Bay.

Keywords: fish population, otolith shape analysis, Bhattacharya's method, spawning season

Introduction

The marble goby, *Oxyeleotris marmorata* (Bleeker, 1852) is a member of the family Eleotridae and

native to fresh and brackish waters of Southeast Asia. *Oxyeleotris marmorata* is a highly sought-out food fish and has a large market demand domestically and internationally. The yield of wild *O. marmorata* has been decreased drastically due to habitat destruction and overfishing (Cheah et al., 1994). Tavarutmaneegul and Lin (1988) reported successful commercial production of *O. marmorata* of 147,300 juveniles in a year with high survival of 60 to 99% at Non-Sua Fisheries Station in Thailand. Nevertheless, the production of *O. marmorata* reduced drastically in Thailand during the 1990s, resulting mainly from outbreaks of disease (Jiwyam, 2008). The culture currently relies heavily on seed collection from the wild, subsequently, the supply of juveniles, traditionally recruited from natural waters, has become limited and imposes a severe deterrent to grow-out production (Teoh et al., 2019). This species is currently listed as a species of Least Concern on the IUCN Red List (Larson, 2019). However, in Indonesia, it is reported that the species has already been fished almost to the point of population collapse (Herawati et al., 2017).

Conservation actions for wild *O. marmorata* is an urgent requirement, however biological information required for monitoring wild *O. marmorata* populations is limited (Lestari et al., 2019). In fisheries management and assessment, population is the fundamental biological unit and population identification in the species is very important (Reiss et al., 2009). Only with understanding the geographical variation and reproductive biology, it is possible to effectively manage and subsequently conserve endemic fish populations (Al-Saleh et al., 2012). Otolith morphometrics have been shown to provide a practical basis for population identification and subsequent fisheries management (Keating et al., 2014, Libungan et al., 2015, Vasconcelos et al., 2018).

During fish market surveys in Kota Kinabalu, Sabah, Malaysia, *O. marmorata* was found often caught by small scale local fishermen using various fishing gears, mainly bottom gill nets, throughout the year in Likas Bay, Kota Kinabalu. Likas Bay is a marine bay with an average seawater salinity of 32.0 ± 2.1 ppt (Azad & Jinau, 2020). The objectives of this study are to identify the population structure of the marine *O. marmorata* and to determine their reproduction cycle. The findings obtained in this study provide basic biological parameters of *O. marmorata* populations in Likas Bay.

Materials and Methods

Study area and fish specimen collection

Likas Bay, faces the South China Sea (Figure 1), has shallow water and is partly fringed with mangrove forest and sandy beach. The shallow waters and man-made beaches of the Bay are popular among recreational visitors and serve as an important fishing ground for small-scale fishers. During the sampling period, we procured 147 specimens (male: female sex ratio was 73: 74) caught by the small-scale fishers. Systematic samplings were not conducted as the landings of *O. marmorata* in Likas Bay

were irregular. As such, there were no specimens obtained between February and August 2018. Additionally, dead specimens of *O. marmorata* were procured from the Kota Kinabalu fish market from January 2018 to November 2019.

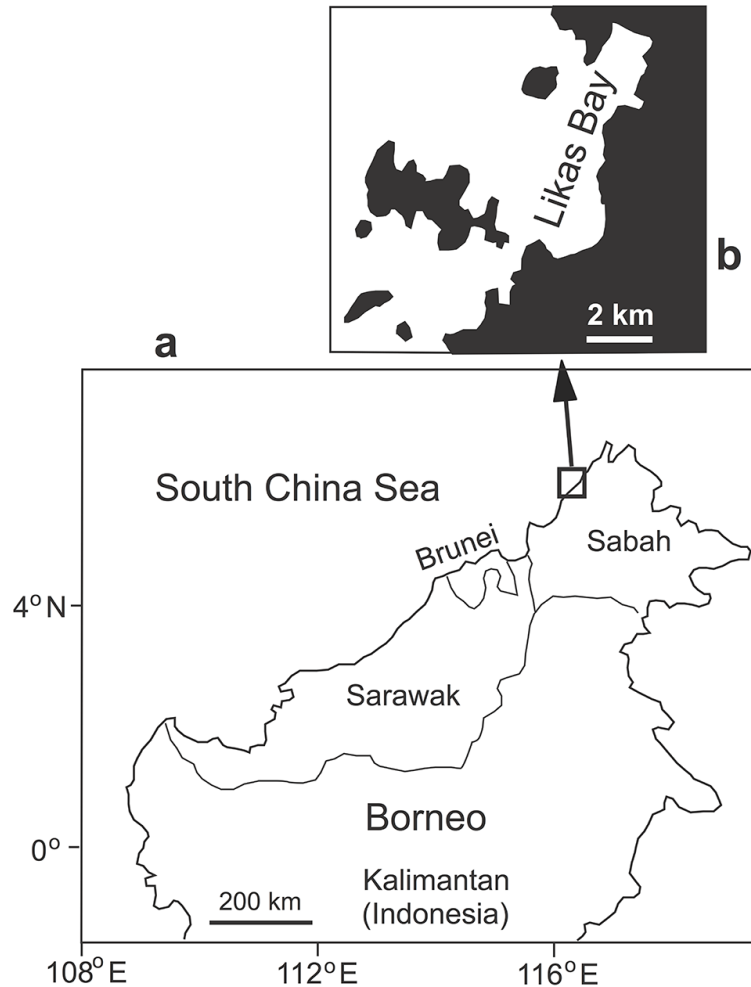


Figure 1. Map showing the location of Borneo (a) and Likas Bay (b), Sabah, Malaysia.

The body size of the fish specimens ranged from 328 mm in total length (TL) and 443 g in body weight (BW) to 594 mm TL and 3,264 g BW. After photographing the urogenital papilla and weighing the body weight to the nearest 1 g, the body cavity of each specimen was dissected, and the intact gonads were photographed. The gonads and alimentary canal contents were weighed to the nearest 0.1 g with a digital balance (TANIT, Tokyo, Japan). As there is no difference in gonad development between sites within the same gonad (Smith & Walker 2004), a piece of frontal part of gonads was sampled and preserved in 10% formalin for histological examination of the gonadal developmental stages.

Otolith collection and otolith shape analysis

The fish head was ventrally dissected and the sagittal otoliths of 147 specimens were removed. The sagittae were photographed with a smartphone camera (Huawei Nova 7i, Huawei, China) and the length and width of the right sagitta were measured (Figure 2) with a digital caliper (Shinwasokutei, Sanjo, Japan) to the nearest 0.01 mm. The measurement was done three times for one otolith and the median value was adopted. Then the ratio of width to length of the otolith was calculated. This ratio is the curvature index of the otolith; a bulky otolith has a large curvature index and vice versa.

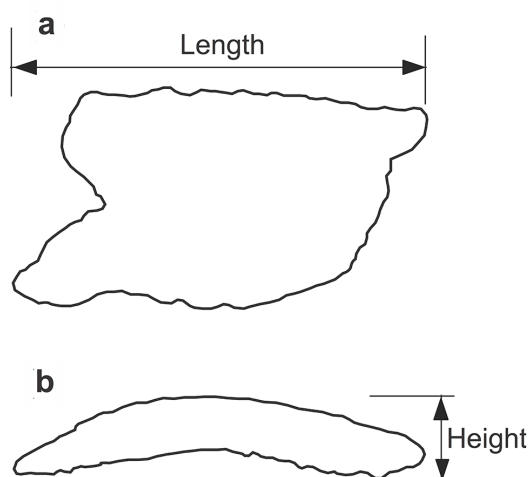


Figure 2. Illustration of dimensions of sagittal otolith of *Oxyleotris marmorata* showing measurement of length and width.

The frequency distribution of a morphometric character in a biological population is usually skew and polymodal; in many cases, a mixture of normal components. Bhattacharya's method (Bhattacharya, 1967) is simple and useful for separating the normal components. Method, $y(x)$ denotes the observed frequency in the class with x as its mid-point and h denote the class interval. The log differences, $\Delta \log y = \log y(x + h) - \log y(x)$, are plotted against x on an ordinary graph paper, and regions where the graph looks like a straight line with negative slope was determined. The number of straight lines of such regions is the number of normal components (Bhattacharya, 1967).

Age estimation

Three vertebrae, from 3rd to 5th vertebrae of each specimen, were sampled and preserved in 10% formalin for the later counting of the vertebral rings. Clear rings on the cleaned wet vertebrae can be observed by naked eye without sectioning or staining (Figure 3). The rings on the vertebrae indicate

the annuli, and age was estimated by counting the number of opaque rings observed on the inner surface of the cones in three vertebrae for each specimen. The counting was facilitated by placing a vertebra vertically on a slide glass and illuminating the vertebra upwards from the bottom with a light guide (LS-JHS. Sumita Optical Glass Inc., Saitama, Japan). The counting was done without the information on the fish size to avoid nuisance factors in the counting.

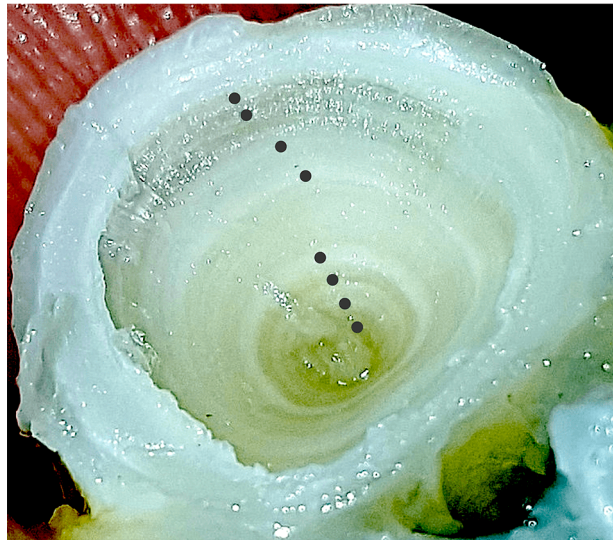


Figure 3. Vertebra from an interpreted 8 years old *Oxyleotris marmorata*. Closed circles represent opaque rings on the inner surface of the cones.

Gonad development analysis

The ovary development stages were determined macroscopically and histologically. For the histological study, the gonad samples were dehydrated in ascending series of ethanol, embedded in paraffin, sectioned in 7 μm thick, and stained with haematoxylin and eosin. The gonadosomatic indices (GSI) was calculated as the percentage (%) ratio between gonad weight and body weight which did not include the weight of the alimentary canal contents and gonads.

Results and Discussion

Otolith shape analysis and population structure

The results of the otolith curvature analysis by the Bhattacharya's method is shown in Figure 4. The graph of logarithmic differences of the class-frequencies against the mid-point of the classes shows two straight lines with negative slope (Figure 4). This shows that the frequency distribution of the curvature index is a mixture of two normal components; indicating the presence of two different populations in Likas Bay.

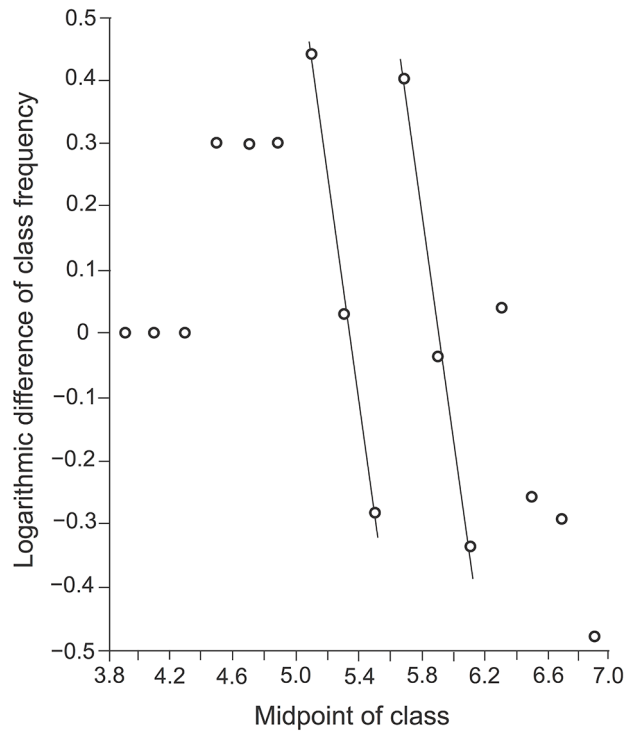


Figure 4. Graph of logarithmic differences of the class-frequencies against the mid-point of the classes. Two straight lines with negative slope indicate two normal components.

Ha et al. (2011) examined mitochondrial DNA of 85 *O. marmorata* specimens sampled from 14 locations in Southeast Asia and reported three genetically differentiated populations, i.e. Ayutthaya in Thailand, Dong Nai in Vietnam, and Sabah in East Malaysia. As geographically close samples were pooled and treated as a single population in their study, fine population structure was not detected.

Age structure of sample fish

There was no difference in the three counting of the vertebral rings in all specimens. The age of *O. marmorata* sampled ranged from 5 to 14 years. The age frequency distribution was skewed and polymodal with the first peak at 6 years and the second peak at 8 years (Figure 5). This age frequency distribution may not accurately represent the true population structure, as smaller specimens were likely under-sampled due to the size selectivity of the gill nets. Two specimens of 530 mm TL and 670 mm TL had a clear net-mark at the posterior part of the head, indicating a large mesh size of the gill net used.

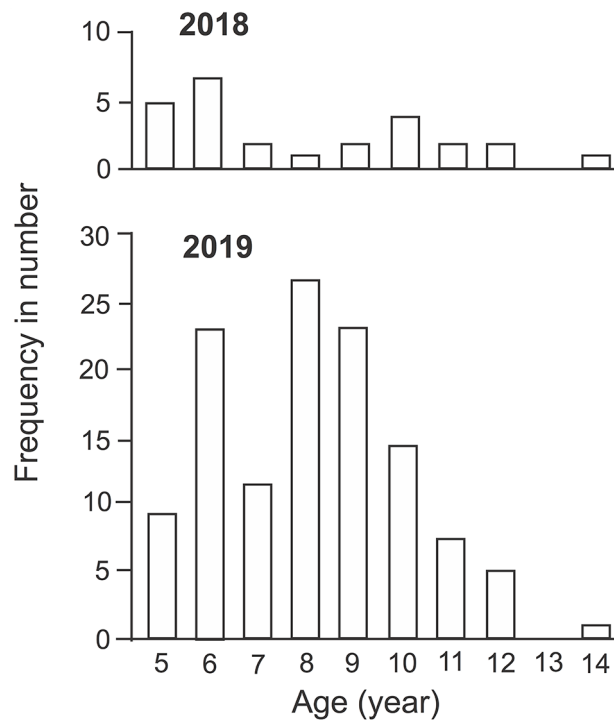


Figure 5. Frequency distribution of age of *Oxyeleotris marmorata* sampled in 2018 ($n = 26$) and 2019 ($n = 121$).

Ovary development and spawning season

The maturation stages were determined only for females since the testes were very small (GSI ranged from 0.03 to 1.76) and mature ones were not recognized macroscopically. Generally, there are three stages of the ovary development: maturing, mature and spent. Figure 6 shows photographs and microphotographs of histological sections of ovaries from two specimens sampled in January 2018. The mature ovary is yellowish in colour, distinctly bulging and large (Figure 6a) and filled with well-developed mature oocytes (Figure 6b) while oocytes did not run out when light pressure was applied. The spent ovary was slender, bloodshot and flaccid (Figure 6c). In the histological sections, spent ovaries contained scattered granular atretic oocytes and post ovulatory follicle (Figure 6d). Unspawned large ova and small ova were not visually recognized in all spent ovaries.

While the GSI is commonly used for staging gonadal development, these may be biased when samples of fish of different body weight are compared, as pointed out by West (1990). Indeed, GSI does not show the presence of spent ovaries. GSI of spent females did not give quantitative information on the spawning season. Therefore, the spawning season was estimated based on the presence of females with mature ovary or spent ovary. Figure 7 shows the distribution of GSI of female *O. marmorata* by sampling month. Specimens with mature ovaries were sampled only in September 2018, and in January and February 2019. Specimens with spent ovaries were sampled in January and September 2018, and in January, February and September 2019.

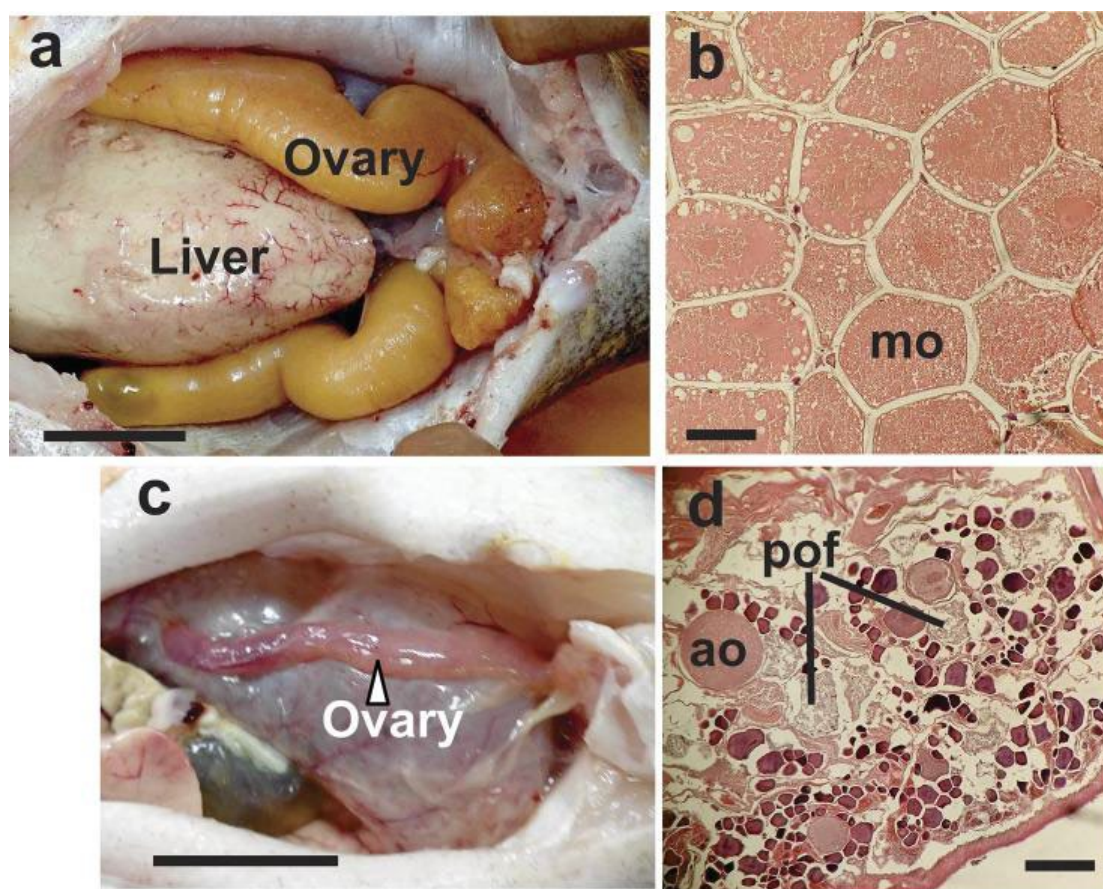


Figure 6. Photographs of ovaries (a & c: scale bar, 2 cm) and microphotographs (b & d: scale bar, 0.2 mm) of histological sections of ovaries of mature female [(a) and (b)], and spent female [(c) and (d)] of *Oxyeleotris marmorata*. (a) and (b), sampled on 26 September, 2018, BW 2,325 g, GW 52.4 g, GSI 2.254; (c) and (d), sampled on 11 January, 2018, BW 945 g, GW 2.6 g, GSI 0.275; mo, mature oocyte; ao, atretic oocyte; pof, post ovulatory follicle.

Based on the appearance of specimens with the mature or spent ovaries, it is concluded that the spawning of *O. marmorata* in Likas Bay took place twice, in January-February and September in 2018 and 2019. Tavarutmaneegul and Lin (1988) reported that each female spawned at least three times annually in the earthen ponds at Nong-Sua Fisheries Station in Thailand. In the present study, the spent ovaries of the samples did not contain unspawned large ova which indicates that *O. marmorata* are single spawners. Therefore, it is concluded that the spawners are from two different populations. Even though the two populations are sympatric in the small bay (insufficient spatial isolation), the different spawning seasons with a half-year time interval (sufficient temporal isolation) might be sufficient to prevent gene flow between the two populations and hence enable self-sustaining each other.

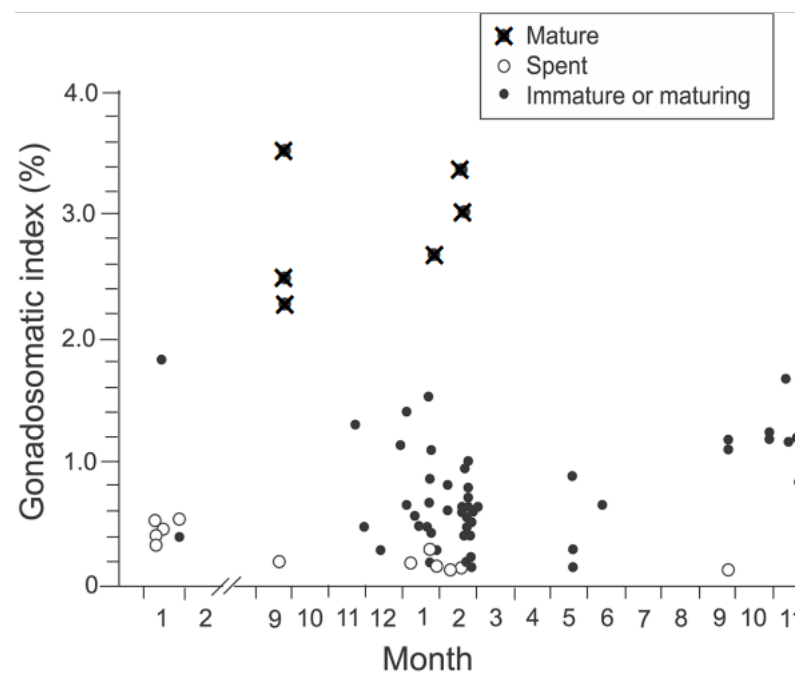


Figure 7. Distribution of gonadosomatic indices (GSI) of female *Oxyeleotris marmorata* by sampling month.

It is reported that the sexes of freshwater *O. marmorata* are distinguishable by the morphology of the urogenital papilla; the males have a small, flat triangular structures while the females have a large finger-shaped protrusion which become reddish especially at the tip during the spawning season (Tavarutmaneegul & Lin 1988; Cheah et al., 1994; Idris et al., 2012). However, this method of sex identification was not able to apply to the marine populations of *O. marmorata*. We found 20 out of 73 males with red urogenital papilla (Figure 8). Thus, the biological traits relevant to reproduction of marine populations of *O. marmorata* are different from those of freshwater populations.

The inhabitation of marine *O. marmorata* is not restricted to Likas Bay, but appears to be in the wider region in Borneo. Hatchery-produced *O. marmorata* juveniles tolerate a wide range of water salinity up to 30 ppt in the laboratory, although growth performance and survival are low compared to rearing in 0 and 10 ppt (Darwis et al., 2008). Recreational fishers occasionally catch *O. marmorata* in the coastal waters of Sarawak, Borneo (G. Kawamura, personal observation). Such capture information and population study are important for the better understanding of *O. marmorata* ecology.

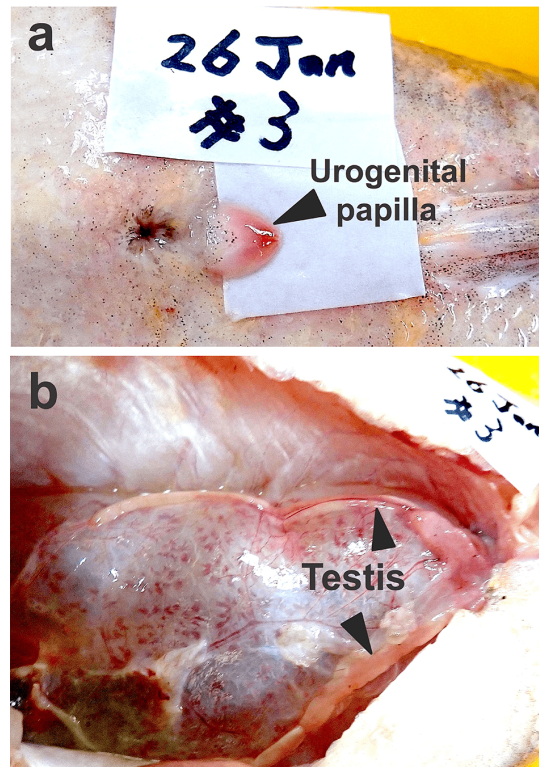


Figure 8. Photographs showing the urogenital papilla (a) and the testis (b) from the same male *Oxyeleotris marmorata*.

Conclusion

This study provided the first biological data on the *O. marmorata* collected from the marine ecosystem of Likas Bay, Sabah, Malaysia. The population structure of this marine stock determined by otolith shape analysis by the Bhattacharya's method indicated two different populations. The specimens with mature or spent ovaries were found in samples collected in January–February and September, showing two spawning seasons. The age of each specimen was estimated by counting the vertebral annuli. The minimum size and age of the spawner was 338 mm TL, 499 g BW and 5 years old. The findings obtained in this study provide basic biological parameters for the conservation of marine *O. marmorata* populations in Likas Bay.

Author Declaration on Animal Research Studies

The present study adhered to the Researcher Guideline outlined in the Code of Practice for the Collection, Care, and Use of Animals for Scientific Purposes established by Universiti Malaysia Sabah.

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Refining of Water Masses at Sarawak Waters, Malaysia

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Abstract

Water mass identification is essential to understand the dynamics of water circulation. The waters off Sarawak are influenced by the northeast and the southwest monsoons. We studied the physical oceanography of the South China Sea along these waters, from data collected during the Prime Marine Scientific Expedition 2009. The expedition was organized by the Malaysian Ministry of Science, Technology, and Innovation through the National Oceanography Directorate. The physical properties of seawater were obtained using the 'Conductivity-Temperature-Depth' (CTD) profiler from the ocean surface to a water depth of 523 meters. Measurements were recorded from 62 stations, with the furthest offshore distance being approximately 471 kilometers from the nearest point of Sarawak's mainland. The aim of this study is to identify the mass features of Sarawak waters. We employed the T-S diagram and refined the water masses based on previous studies conducted in the South China Sea. The constructed vertical salinity and temperature profiles showed that salinity increases towards the sea bottom, whereas temperature decreases as water depth increases. Five water masses were identified off Sarawak waters, namely Continental Shelf Water (CSW), Open Sea Water (OSW), Maximum Salinity Water (MSW), Permanent Thermocline Water (PTW), and Water Mass 1 (WM1). WM1 is derived from the mix of OSW and MSW masses. Results of this study are useful for studies in primary productivity and the distribution of marine resources in these waters.

Keywords: Vertical profiles, T-S diagram, water mass, Sarawak waters, South China Sea

Introduction

The water characteristics of the South China Sea (SCS) area are subject to seasonal monsoons.

From November to March, the Northeast monsoon dominates the SCS region, causing strong northeasterly wind stress in Malaysian waters. The Southwest monsoon dominates this area from April to August, driving a northward coastal current off the east coast of Malaysia (Akhir, 2012). During this monsoon, the surface current starts flowing northeasterly from the west coast of Borneo Island along the coast of Sarawak (O'Neil & Eason, 1982). Siripong (1984) stated that the surface current during the transition period between the Northeast and Southwest monsoons in May was weaker and varied in direction compared to ordinary monsoons. Therefore, the vertical and horizontal structures of the water properties in the SCS may change during different seasonal monsoons. Vertical structures off Sarawak waters were discussed by Manson et al. (2018).

The properties of seawater, such as temperature and salinity, have been used as guidelines or passive tracers to provide direct pathways of water masses (Arsad & Akhir, 2013). A water mass in the ocean is defined based on water temperature (°C) and salinity (PSU) characteristics. A water mass is an identifiable body of water with a common formation history that has a distinctive narrow range of temperature and salinity from surrounding waters (Marghany, 2012). Song (1994) explained that a single water mass can occupy a particular oceanic region, but at the same time, several water masses can be present at an oceanic location as the water masses mix and spread across that area. Each water mass is first identified by its water characteristics, followed by observing the ocean process that created that specific characteristic. It is then necessary to compile temperature, salinity, and water depth to understand the differences in water masses. One of the standard methods for water mass identification is based on a temperature-salinity (T-S) diagram (Marghany, 2012). According to Dippner and Loick-Wilde (2011), definitions of water masses for areas with high variability of ocean parameters are useful to understand the complexity of the biological system, such as plankton in upwelling areas.

Akhir (2012) identified higher phytoplankton density in the coastal areas of Sarawak during the southwest monsoon. A comparison of prokaryotic communities in the surface waters of the eastern South China Sea (SCS) revealed that surface microbial community patterns in Sarawak waters exhibited a more distinctive composition and metabolism (Song et al., 1994). This might be related to water mixing and the properties of seawater. However, the study of the physical characteristics of Sarawak waters is limited to understanding the vertical and horizontal features of water, such as water mass definition.

Previously, the study of water masses on the Sarawak coast was conducted by Saadon et al. (1998a) during the Southeast Asian Fisheries Development Centre (SEAFDEC) cruise held in 1997. Another study on temperature and salinity profiles off Sarawak waters was discussed by Saadon et al. (1988b) during the Matahari Expedition in 1985. Manson et al. (2018) elaborated on the temperature and salinity profiles of Sarawak waters, defining the water properties at different depths. These water properties were analyzed for water mass definition and classification based on studies by Qu et al. (1999; 2000) in the western SCS, Rojana-Anawat et al. (2001), Dippner and

Loick-Wilde (2011) in Southeast Asia waters, and Arsad and Akhir (2013) in Sabah waters. Thus, the objective of this study is to redefine the water masses based on the previous study done in SCS and to identify the water mass features in Sarawak waters, Malaysia.

Methodology

Study Area

The Prime Marine Scientific Expedition 2009 (PMSE '09) took place over 44 days between June and July 2009 in the waters of Malaysian Borneo, encompassing the South China Sea (SCS), the Sulu Sea, and the Celebes Sea. The expedition aimed to collect coastal and offshore oceanographic data and was organized by the Ministry of Science, Technology, and Innovation (MOSTI) through the National Oceanography Directorate (NOD), in close collaboration with the National Hydrographic Centre, Royal Malaysian Navy (RMN), and local universities. During the expedition, physical oceanographic data collected in the SCS within Sarawak waters were analyzed to identify water masses at depths less than 500 m, covering a survey area from 2.8737°N to 4.4446°N and 109.9034°E to 113.3421°E (Figure 1).

Water temperature and salinity were measured at 62 stations located approximately 50 km to 471 km off Sarawak's mainland (Figure 1). In this study, sampling stations closer to the shoreline of Sarawak waters, with a water depth of less than 50 m, are considered shallow waters, while those located further offshore with water depths greater than 50 m are designated as deep water (Manson et al., 2018). Measurements were conducted during the southwest monsoon in June 2009, using an SBE 19Plus Conductivity-Temperature-Depth (CTD) profiler, recording temperature and salinity data from the sea surface down to 523 m water depth.

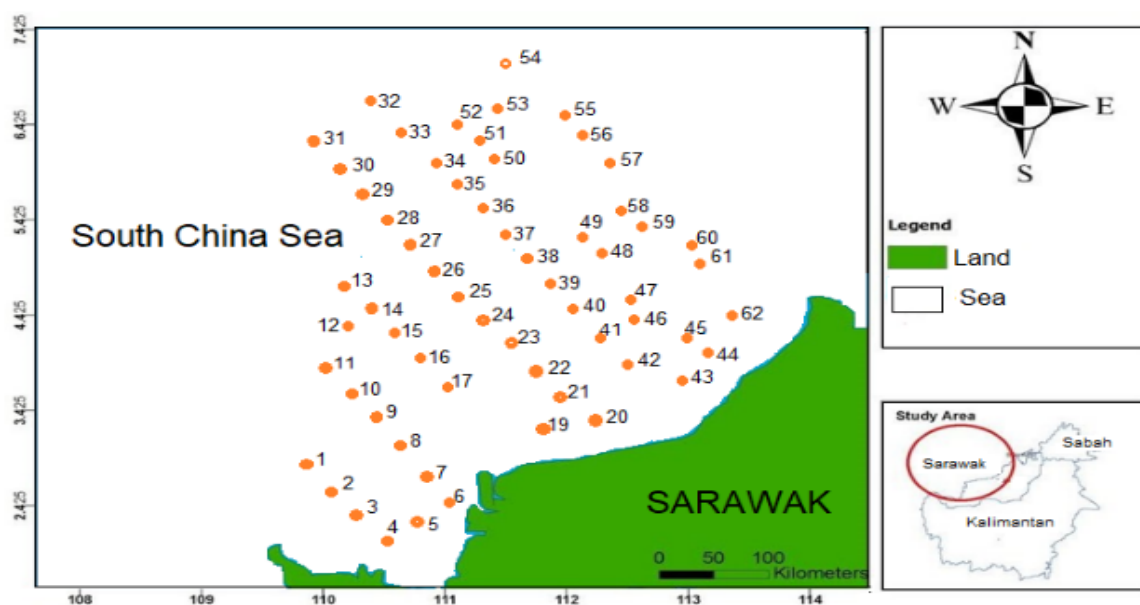


Figure 1. Location of 62 Stations at Sarawak Waters

Data Analysis

The temperature and salinity data were compiled using Microsoft Excel to create temperature-salinity (T-S) diagrams, identifying the water characteristics of the study areas. T-S diagrams were also employed to mark the water mass distribution based on specific water mass classifications in the SCS published by Qu et al. (1999; 2000), Rojana-Anawat et al. (2001), Dippner and Loick-Wilde (2011), and Arsad and Akhir (2013).

Water mass distribution in the SCS, particularly along the Philippine coast, was identified based on the relationship between potential temperature versus salinity and dissolved oxygen versus salinity data (Qu et al., 1999; 2000). Qu et al. (2000) recognized water masses in this area as Tropical Surface Water (TSW), North Pacific Tropical Water (NPTW), South Pacific Tropical Water (SPTW), and North Pacific Intermediate Water (NPIW). Characteristics of the Antarctic Intermediate Waters (AAIW) were also found in this region as part of the Indonesian throughflow (Wrytki, 1961). Rojana-anawat et al. (2001) classified water masses along the Vietnamese waters of the western SCS as Deep Water (DW), Permanent Thermocline Water (PTW), Maximum Salinity Water (MSW), Open Sea Water (OSW), Seasonal Thermocline Water (STW), Northern Continent Shelf Water (NCSW), and Southern Continental Shelf Water (SCSW). Dippner and Loick-Wilde (2011) redefined water mass characteristics in SCS as DW, PTW, MSW, OSW, and Mekong/Gulf Thailand Water (MKGTW), while Arsad and Akhir (2013) discovered two local water mass characteristics in east SCS: the upper layer of Sabah waters known as Continental Shelf Water (CSW) and OSW. These water mass definitions were done by comparing the temperature and salinity values to the measured data from the CTD. The range of temperature and salinity values were overlaid with the T-S diagram from the expedition to redefine and determine the specific water masses of Sarawak waters. The temperature and salinity range of the earlier water mass definitions in the SCS were also adjusted to present information specifically for this study.

Results and Discussion

Definitions and Reconstruction of Water Mass in the South China Sea (SCS)

The water masses in the SCS are influenced by their location and sources of water. For instance, the Mekong/Gulf Thailand Water (MKGTW) mass is primarily affected by the Mekong River at Gulf Thailand. The uppermost layer of the SCS typically constitutes a surface layer with uniform hydrographic properties, known as the surface mixed layer (Song et al, 1994). The construction of the T-S diagram for Sarawak waters reveals that salinity vertical profiles increase towards the sea bottom, while temperature decreases as water depth increases (Figure 2).

Water mass definitions identified by Qu et al. (1999) and Qu et al. (2000) along the Philippine coast of the SCS were plotted on the constructed T-S diagram for Sarawak waters (Figure 3a). However, none of these water mass definitions fit this T-S diagram. Nevertheless, the three water masses (OSW, MSW, and PTW) identified by Rojana-anawat et al. (2001) are fitted to

the T-S diagram (Figure 3b).

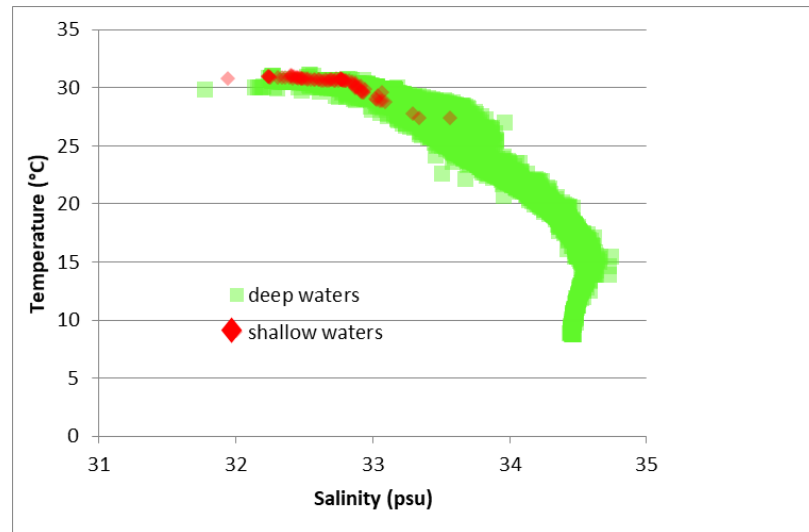
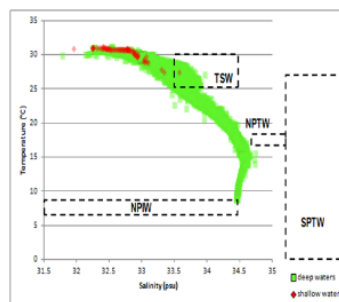
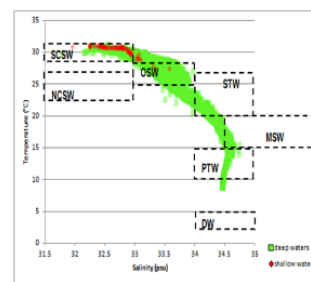


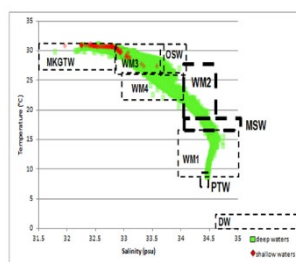
Figure 2. T-S diagram of Sarawak waters



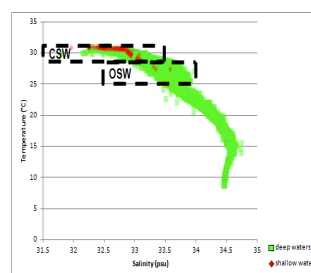
a: Water mass classification by Qu et al. (1999; 2000)



b: Water mass classification by (Rojana-anawat et al. (2001)



(c): Water mass classification by Dippner & Loick-Wilde (2011)



(d): Water mass classification by Arsad & Akhir (2013)

Figure 3. Water mass classification based on previous studies carried out at SCS; Tropical Surface Water (TSW), North Pacific Tropical Water (NPTW), South Pacific Tropical Water (SPTW) and North Pacific Intermediate Water (NPIW), Northern Continent Shelf Water (NCSW), Seasonal Thermocline Water (STW), Permanent Thermocline Water (PTW), Deep Water (DW), Continental Shelf Water (CSW), Open Sea Water (OSW), Water Mass (WM), Maximum Salinity Water (MSW), Mekong/Gulf Thailand Water (MKGTW) and Southern Continental Shelf Water (SCSW).

The water masses (WM1 and MSW) identified by Dippner and Loick-Wilde (2011) and the water masses (CSW and OSW) reported by Arsad and Akhir (2013) all fit well with the T-S diagram of Sarawak waters (Figure 3c-d). Based on the redefinition of water masses within the SCS, five water masses (CSW, OSW, WM1, MSW, and PTW) were matched with the water masses of Sarawak waters during the Southwest monsoon in June 2009 (Figure 4).

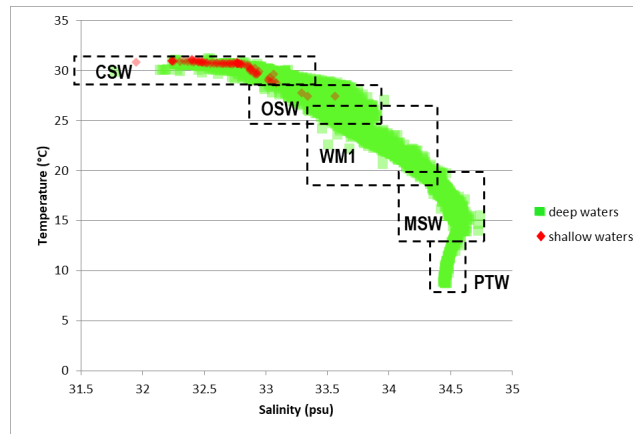


Figure 4. T-S diagram of all CTD casts in Sarawak waters with labeled classification of water mass identified in the SCS. The dotted rectangular labeled on the T-S ranges diagram is the best match to describe the water masses off Sarawak waters.

Water Mass Definitions and Reconstruction of Sarawak Waters

The water characteristics and newly redefined water mass characteristics in the SCS are summarized by Arsad & Akhir (2013) and Dippner & Loick-Wilde (2011). The layer of the sea is divided into surface, middle, and bottom layers (Table 1). The upper 50 m water depth is known as the surface layer. This layer is covered by two distinct water masses: the CSW and the OSW. The CSW is present in Sarawak waters, with temperature and salinity ranging between 29°C to 31°C and 27 psu to 33.5 psu, respectively (Arsad & Akhir, 2013). The OSW is found in deeper waters between 57 m to 75 m. However, traces of these water masses are also found in shallow waters (for example, St. 8 starting from 37 m depth) with temperature and salinity ranging from 25°C to 29°C and 32.9 psu to 34.1 psu, respectively.

The middle layer of the SCS, originally classified by Dippner & Loick-Wilde (2011) as Water Mass 2 (WM2) (Table 1), is redefined as the middle layer for Sarawak waters, known as WM1. This water mass is derived from the mixing of OSW and MSW water masses, with a wider range of salinity (33.4 to 34.4 psu) compared to WM2 with a salinity range of only 34.1 to 34.6 psu. However, both water masses have a similar temperature range from 19 to 28°C.

The bottom layer water masses of the SCS are identified as MSW and PTW (Table 1). The MSW water mass is characterized by a temperature and salinity range of 13°C to 20°C and 34.2 psu to 34.8 psu, respectively. The PTW is usually located between the water masses of MSW and DW (Dippner & Loick-Wilde, 2011). It is detected when an obvious thermocline formation occurs

in the T-S diagram at deeper waters (Figure 2). There are increases in temperature and salinity for PTW at Sarawak waters compared to the PTW defined by Dippner & Loick-Wilde (2011) due to a significant thermocline formation in the T-S curve (Figure 3c). The new definition of PTW is located below the MSW layer where the temperature ranges between 8°C and 13°C, and salinity ranges from 34.4 psu to 34.7 psu. The minimum temperature of 8°C shows that the PTW is located in the deeper waters of Sarawak coastal areas.

Table 1. Redefinition of water mass and its characteristics in Sarawak waters based on previous studies in the SCS.

Ocean layer	Water mass	Temperature (°C)	Salinity (psu)	Source	Water mass re-definition	Redefined temperature (°C)	Redefined salinity (psu)
Surface layer	CSW	29-31	27.0-33.5	Arsad & Akhir (2013)			
	OSW	25-29	32.5-34.0	Arsad & Akhir (2013)	OSW	25-29	32.9-34.1
Middle layer	WM2	19-28	34.1-34.6	Dippner & Loick-Wilde (2011)	WM1	19-28	33.4-34.4
Bottom layer	MSW	17-19	>34.3	Dippner & Loick-Wilde (2011)	MSW	13-20	34.2-34.8
	PTW	7-9	34.4-34.5	Dippner & Loick-Wilde (2011)	PTW	8-13	34.4-34.7

The bottom layer water masses of the SCS are identified as MSW and PTW (Table 1). The MSW water mass is characterized by a temperature and salinity range of 13°C to 20°C and 34.2 psu to 34.8 psu, respectively. The PTW is usually located between the water masses of MSW and DW (Dippner & Loick-Wilde, 2011). It is detected when an obvious thermocline formation occurs in the T-S diagram at deeper waters (Figure 2). There are increases in temperature and salinity for PTW at Sarawak waters compared to the PTW defined by Dippner & Loick-Wilde (2011) due to a significant thermocline formation in the T-S curve (Figure 3c). The new definition of PTW is located below the MSW layer where the temperature ranges between 8°C and 13°C, and salinity ranges from 34.4 psu to 34.7 psu. The minimum temperature of 8°C shows that the PTW is located in the deeper waters of Sarawak coastal areas.

The salinity profile of Sarawak waters increases with water depth. The maximum salinity at a water depth of approximately 500 m from the sea surface is 34.8 psu (Manson et al., 2018). The overlapping area, marked as rectangles of the OSW and MSW, was identified at the middle layer of SCS (Figure 4). In this study, the occurrence of mixing water masses is defined as Water Mass 1 (WM1), where the temperature and salinity range from 19°C to 28°C and 34.4 psu to 34.7 psu, respectively (Table 1). Therefore, no DW was detected, as defined by Rojana-Rawat et al. (2001). The DW usually exists at a water depth of more than 900 m. In this study, the temperature

and salinity data were measured only down to a maximum water depth of approximately 523 m.

Redefinition of Water Masses in Sarawak Waters

Water masses such as CSW and OSW, as defined by Arsad and Akhir (2013) in Sabah waters, also occupy the uppermost layer of Sarawak waters, characterized by a temperature range of 29°C to 31°C and a salinity range of 27 psu to 33.5 psu (Table 1). These water characteristics perfectly align with the surface layer of the T-S diagram of Sarawak waters. It is suggested that a similar water mass is found in the surface waters of both Sabah and Sarawak. The newly defined OSW in Sarawak waters shares the same temperature range of 25°C to 29°C and a salinity range of 32.9 psu to 34.1 psu. The OSW water mass dominates Sarawak waters and has been consistently defined in previous studies within the SCS area. For instance, Rojana-Anawat et al. (2001) characterized the temperature range as 25 to 29°C and a salinity range of 33 to 34 psu in Vietnamese waters. It was later redefined by Dippner and Loick-Wilde (2011) in the same study area (Vietnamese waters), with a slight increase of 1°C in the temperature range and a smaller range of salinity. This study suggests that OSW in Sarawak waters is more saline compared to the water characteristics in Sabah as defined by Arsad and Akhir (2013).

The water mass at the middle layer of Sarawak waters exhibits similar characteristics to the MSW and PTW defined by Dippner and Loick-Wilde (2011). It likely originates from the Northwest Pacific Ocean, where the MSW and PTW are named as NPTW and PTW water masses, respectively, on the Philippine coast (Qu et al. 2000). The MSW was initially distinguished by Rojana-Anawat et al. (2001) with a temperature range of 15 to 20°C and a salinity range of more than 34.5 psu. However, according to Dippner and Loick-Wilde (2011), the temperature range set by Rojana-Anawat et al. (2001) is unclear, and the salinity range is too small. The MSW in Sarawak waters is distinguished by a redefined temperature and salinity range of 13 to 20°C and 34.2 to 34.8 psu, respectively. The temperature and salinity range are not as wide as the MSW in Vietnamese waters.

Conclusion

The surface layer in the study of Sarawak waters aligns best with the water characteristics defined by Arsad and Akhir (2013), while the middle and bottom layers closely resemble the water characteristics defined by Dippner and Loick-Wilde (2011). Five types of water masses (CSW, OSW, MSW, PTW, and WM1) have been identified in Sarawak waters. WM1 represents a mixed water mass of OSW and MSW detected between the OSW and MSW layers. However, both Qu et al. (1999; 2000) and Rojana-Anawat et al. (2001) generally do not fit with the T-S diagram of Sarawak waters, where the T-S range is distinctly different compared to these findings. The number of water masses in Sarawak waters could potentially be higher with further measurements towards the sea bottom. Water masses may also vary with data measurements

carried out during different seasonal monsoons, such as the northeast monsoon. It is suggested that further studies on water mass definition be conducted during different seasonal monsoons such as intermonsoon and northeast monsoon. A comprehensive understanding of water masses would enhance biological knowledge, including primary productivity and the distribution of marine resources in this area.

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The First Record of Sona Sea Catfish *Pseudosciades sona* (Hamilton 1822) in Sabah, Malaysian Borneo, on the Basis of Morphological and Molecular Data

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Abstract

The Sona Sea Catfish *Pseudosciades sona* is widely distributed across the Indo-West Pacific, including Malaysia. In this paper, we report the first record of the species from Sabah, based on morphological and molecular data. The single specimen of *Pseudosciades sona* was obtained during a field survey, from the fish market in Beluran, Sabah. The DNA of the specimen has been sequenced and data was published in elsewhere. The species is diagnosed by a combination of morphometric characteristics, including 10–12 pectoral-fin rays, long head (27.59%–31.85% of SL), long snout (28.89%–35.86% of HL), wide mouth (58.04%–65.72% of HL), eye diameter (12.90%–15.75% of HL), distance between below the eye to the bottom of the head (18.95%–24.57% of HL), distance between behind the eye and the margin of operculum (55.75%–60.32% of HL), bony interorbital width (53.67%–60.03% of HL), long bases of dorsal-fin (5.21%–6.18% of SL), anal-fin (13.12%–16.64% of SL), pectoral-fin (5.21%–6.18% of SL), and pelvic-fin (4.14%–5.34% of SL). This paper describes the morphological features of this species, with a brief discussion on catfish conservation and its significance as a fishery resource in Malaysia.

Keywords: Ariidae, *Pseudosciades sona*, new record, Sona Sea Catfish, Sabah

Introduction

The catfish order Siluriformes comprises 41 families, with 512 genera and 4267 species (Van der Laan et al., 2024). Catfishes are distributed circumglobally, in both tropical and temperate waters, and are recorded in marine, estuarine and freshwater ecosystems. Apparently, only 13 families under the suborder Siluroidei can be found in the Western Central Pacific (Van der Laan et al., 2024).

The families Ariidae, along with Bagridae and Siluridae are the three most important sea catfishes in the Western Central Pacific (Froese & Pauly, 2023). Of the three Ariidae subfamilies

which are Ariinae, Bagreinae and Galeichthyinae, only members of subfamily Ariinae have been recorded in Southeast Asia, while the latter two recorded in Africa and the Americas (Kailola, 2004; Acero & Betancur-R, 2007; Marceniuk & Menezes, 2007; Kulongowski, 2010; Matsunuma et al., 2011; Jalal et al., 2012; Marceniuk et al., 2012; Kottelat, 2013; White et al., 2013, Yoshida et al., 2013; Kimura et al., 2015; Abdurrahman et al., 2016; Marceniuk et al., 2017; Marceniuk et al., 2023). Ariidae is also the most commercially important catfish in Malaysia (DoFM, 2020).

Previous studies on catfishes in Malaysia listed 30 species of Ariidae (Roberts, 1989; Kailola, 1999; Kailola, 2004; Marceniuk & Menezes, 2007; Jalal et al., 2012; Kimura et al., 2015; Abdurrahman et al., 2016). Based on the compiled list, all species have been recorded in Sabah except four species namely *Arius arenarius*, *Hemiarus stormii*, *H. sumatranus*, and *Pseudosciades sona*.

The presence of both *Arius arenarius* and *Hemiarus harmandi* in the country is questionable. *Arius arenarius* was reported from Penang by Kailola (2004). However, in that study, only part of the specimen was referred to. We note that *A. arenarius* is morphologically similar to *A. arius*, thus, with limited specimen used in that study, the presence of *A. arenarius* is likely a misidentification of *A. arius*. As for *H. harmandi* which was recorded from Baram River, Sarawak in 1901, the identification is questionable due to the systematic complexities of the species (Fricke et al., 2024). The 1901 Sarawak (=Baram, River, Borneo) record was originally listed as *Arius brevirostris* (Steindachner, 1901). The taxonomic status of *Arius brevirostris* which was treated as a synonym of *Arius harmandi* by Kailola (2004), but *A. brevirostris* is presently considered as *incertae sedis* (Fricke et al., 2024). In this report, we provide the morphometric and meristic data of *Pseudosciades sona* based on a single Sabah specimen. The specimen was barcoded using the COI gene (Nasihin-Seth et al., 2019). Additionally, we provide some notes on the status of catfish conservation and catfish as a fisheries resource in Malaysia.

Materials and Methods

Here we report the first record of Sona Sea Catfish based on a single specimen collected from Beluran fish market. Beluran is a remote coastal town located at the estuary of the Sapi River, on the northeast coast of Sabah, Malaysia (Figure 1). The specimen is from a larger collection of catfishes collected from a fish biodiversity survey from 2016–2017, where several of the catfish collections were sequenced for their DNA study (Nasihin-Seth et al., 2019). The fish survey included field sampling of fresh specimens purchased and collected from fish markets and fish landing sites throughout Malaysia. *Pseudosciades sona* specimens (vouchers no. ZRC 53351 (1-3), ZRC 50386 (3)) from Lee Kong Chian Natural History Museum (LKCNC) in Singapore were also examined in this study.



Figure 1. Map of Malaysia. The red-dot shows the location of Beluran fish market (5°55'31.5"N 117°38'37.2"E), from where the single specimen of *Pseudosciades sona* was collected from.

Morphometric and meristic data from each specimen examined were taken following Ng (2003) and Kailola (2004). The morphometric data are: standard length (SL) which was measured from the tip of the snout to the base of the caudal fin, total length (TL) measured from the tip of the snout to the tip of the upper caudal-fin lobe, head length (HL), snout length (SnL), mouth width (MW), eye diameter (ED), distance between below the eye to the bottom of the head (EH), distance between behind the eye and the margin of operculum (EO), bony interorbital width (IO), body width (BW), dorsal-fin base (DL), anal-fin base (AL), pectoral-fin base (P1L), and pelvic-fin base (P2L). Meristics data include: dorsal-, anal-, pectoral- and caudal- fin ray counts, and gill-raker counts. The morphological data of two closely-related species, *Arius macronotacanthus* and *Osteogeneiosus militaris* were also taken for comparison (Table 1).

Identification keys to ariid catfish species were based on Ng (2003), Kailola (2004), Marceniuk and Menezes (2007), Betancur-R (2009), and Abdurahman et al. (2016). The specimen, along with other species of catfish specimens collected during the same survey, and examined in this study, were deposited at the marine collection of the Borneo Marine Research Institute (IPMB), Universiti Malaysia Sabah. As indicated in the introduction, molecular work on the single specimen of *Pseudosciades sona* was carried out in a separate work (Nasihin-Seth et al., 2019). The results of the DNA sequence (COI gene of the *Pseudosciades sona* specimen) was submitted to GenBank® (<http://www.ncbi.nlm.nih.gov>), with accession number MN094546, and to the BOLD Systems (<https://www.boldsystems.org/>) with identification number BCCM012-19 (Nasihin-Seth et al., 2019).

Table 1. Meristic and morphometric characters of *Pseudosciades sona* (IPMB-I 170816-08.03, ZRC 53351 (1-3), ZRC 50386 (3)), *Arius macronotacanthus* (IPMB-I 010716-17.05, IPMB-I 010716-17.06, IPMB-I 210916-04.01 – IPMB-I 210916-04.05) and *Osteogeneiosus militaris* (IPMB-I 010716-17.12, IPMB-I 150716-17.11, IPMB-I 120816-17.01, IPMB-I 120816-17.02, IPMB-I 120816-17.03, IPMB-I 120816-17.04, IPMB-I 250816-08.07, IPMB-I 151016-80.04, IPMB-I 151016-80.05, IPMB-I 151016-80.06), as well as *Hemiarus verrucosus* (n=7) and *Hemiarus stormii* (n=4) (Ng, 2003).

	<i>Pseudosciades sona</i> n=5 (Present study)	<i>Hemiarus stormii</i> n=4 (Ng, 2003)	<i>Hemiarus verrucosus</i> n=7 (Ng, 2003)	<i>Arius macronotacanthus</i> n=7 (Present study)	<i>Osteogeneiosus militaris</i> n=10 (Present study)
Total length, TL (mm)	218–301	-	-	167–218	189–349
Standard length, SL (mm)	179–237	147.3–281.5	98.7–267.5	132–188	152–292
Dorsal-fin rays	I, 7	-	I, 7	I, 7	I, 7
Anal-fin rays	15–18	-	9–10	17	18
Pectoral-fin rays	I, 10–12	-	11–12	I, 10	I, 10
Pelvic-fin rays	I, 5–6	-	I, 5	I, 5	I, 5
Gill raker	13	-	15–17	16	10–12
Caudal-fin rays	15–18	-	17	15	14
Head length (HL) / SL (%)	27.16–31.85	28.8–29.6	29.3–31.7	24.33–30.38	23.61–28.12
Snout length (SnL) / HL (%)	28.89–35.86	37.0–39.8	35.2–41.7	27.69–32.95	26.18–32.42
Mouth width (MW) / HL (%)	58.04–65.72	-		51.56–57.08	34.21–49.34
Eye diameter (ED) / HL (%)	12.90–15.75	9.7–11.8	10.6–14.0	18.26–25.54	13.32–18.31
Distance between below the eye to the bottom of the head (EH) / HL (%)	18.95–24.57	-	-	14.34–20.94	7.32–13.73
Distance between behind the eye and the margin of operculum (EO) / HL (%)	55.75–60.32	-	-	49.39–53.23	50.44–62.75
Bony interorbital width (IO) / body width (HL) (%)	53.67–60.03	38.3–39.9	26.6–39.7	46.62–53.38	52.77–63.68
Dorsal-fin base (DL) / SL (%)	9.58–12.86	-	10.7–12.5	10.94–13.89	8.51–10.45
Anal-fin base (AL) / SL (%)	13.12–16.64	13.7–14.9	13.6–17.1	13.24–15.83	14.61–18.1
Pectoral-fin base (P1L) / SL (%)	5.21–6.18	-	-	2.98–5.38	3.80–5.17
Pelvic-fin base (P2L) / SL (%)	4.14–5.34	-	-	3.87–4.89	2.81–4.81

Pseudosciades sona (Hamilton 1822)

[English common name: Sona Sea Catfish]

[Malay common name: Duri Sona]

Pimelodus sona Hamilton, 1822: 172, 376 (type locality: India: estuaries of Bengal; types: NT; Hamilton's unpublished figure reproduced in Britz 2019:Pl. 94).

Bagrus trachipomus Valenciennes in Cuvier and Valenciennes, 1840a: 443 (type locality: India: Bengal; holotype: MNHN A.9346, Ferraris, 2007:55).

Hexanematichthys leptocassis Bleeker, 1861g: 65 (type locality: Malaysia: Penang; syntypes [350–360 mm TL]: not preserved) (Kailola, 2004).

Material examined

IPMB-I 170816-08.03, 237 mm SL, mature female, Beluran fish market, Sabah, Malaysia, 17 August 2016. DNA barcoded (Accession number in GenBank®: MN094546; Identification number in BOLD system: BCCM012-19).

Diagnosis

A species of the monospecific genus *Pseudosciades* with the following combination of characters: Finely granulated head shield, two pairs of palatal tooth patches with smaller, round vomerine (centre) tooth patch while the accessory (outer) tooth patch is bigger, containing conical teeth (Kailola, 1999; Marceniuk & Menezes, 2007). *Pseudosciades sona* (Figure 2) differs from other Ariidae species by having conical and sharp teeth in two palatal teeth patches on each side (the outer patch of palatal teeth is triangular with elongated towards out and much bigger than the inner and round patch) (Figure 3). Previously described as *Hemiarus* species, this characteristic differs from *H. stormii* and *H. verrucosus* that has strong, sharp, and conical teeth in 2 oval palatal teeth patches on each side. However, these 2 palatal teeth patches form a contiguous palatal teeth patch in larger fish (Kailola, 1999; Ng, 2003). These species also differ from *P. sona* by having shark-like snout (Ng, 2003).

The single specimen of *Pseudosciades sona* (IPMB-I 170816-08.03), was recorded with the following combination of morphological characters: dorsal-fin rays 7 with 1 serrated spine; anal-fin rays 16; pectoral-fin rays 12 with 1 spine; pelvic-fin rays 5, adipose fin with dark margins.



Figure 2. *Pseudosciades sona*, IPMB-I 170816-08.03, 237 mm SL, mature female, Beluran fish market, 17 August 2016 (not to scale).

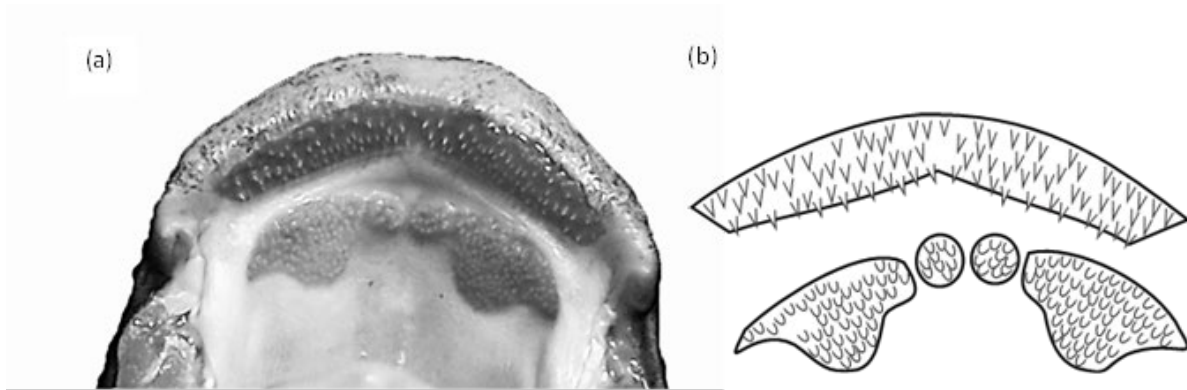


Figure 3. An image (a) and an illustration (b) of the palatal tooth patches of *Pseudosciades sona*, IPMB-I 170816-08.03, 237 mm SL, mature female, Beluran fish market, 17 August 2016 (not to scale).

Remarks

Members of Ariidae are characterized by a deeply forked caudal-fin, presence of an adipose fin, usually three pairs of barbels, a bony shield covering part of the dorsal surface of the head, and unique pattern of the tooth patches on the palate (Kailola, 2004; Marceniuk & Menezes, 2007). Previously, *P. sona* was placed under the genus *Hemiarius* that shares the diagnostic characters of a long adipose-fin base, almost long as the anal-fin base, presence of accessory tooth plates bearing small conical teeth that are transversely elongated and narrow, and two patches of palatal teeth for each side (Marceniuk & Menezes, 2007). Recent paper on the redescription of Ariidae genera has placed this species under a monotypic genus, *Pseudosciades*. This genus is characterised with having moderately long posterior branches of the mesethmoid (bone located at the front skull), apparent

epioccipital (part of back dorsal skull), and slightly concave anterior margin of the nuchal plate. *Pseudo* in Latin means false hence, this genus shares similar characteristics with species of genus *Sciades*. The diagnostic characteristics include a medial groove of neurocranium delimited mainly by frontal bones, anterior and median nuchal plates forming a shield-like structure, and nuchal plate overlapping parietosupraoccipital process (posterior part of skull) (Marceniuk et al., 2023).

The species was only previously recorded elsewhere in Malaysia, precisely from Penang in Peninsular Malaysia (Ng, 2003). Morphological data for *P. sona* remain limited to a few available publications on the species (Kailola, 1999, 2004; Marceniuk & Menezes, 2007; Marceniuk et al., 2023).

The COI gene of the single specimen of *P. sona* reported here (IPMB-I 170816-08.03) was carried out for BLAST in GenBank database in October 2024. Results of that study showed a high similarity (>95%) with the other seven *P. sona* COI sequences in the database. As the published morphological data of *P. sona* is limited, we compared our morphological data to two other closely-related species, *Arius macronotacanthus* and *Osteogeneiosus militaris* (Table 1), which we also recorded in Sabah. In *A. macronotacanthus*, the palatal teeth are conical but sharp, and distributed in a pair of somewhat triangular tooth patches (Figures 4 & 5), whereas in *O. militaris* (Figure 6), the teeth are also conical but sharp, and distributed in a pair of elongated tooth patch, separated by a circular groove anteriorly (Figures 6 & 7). Additionally, *P. sona* has 3 pairs of fleshy barbels (Figure 2), whereas *O. militaris* only has a pair of stiff barbel (Figure 6).

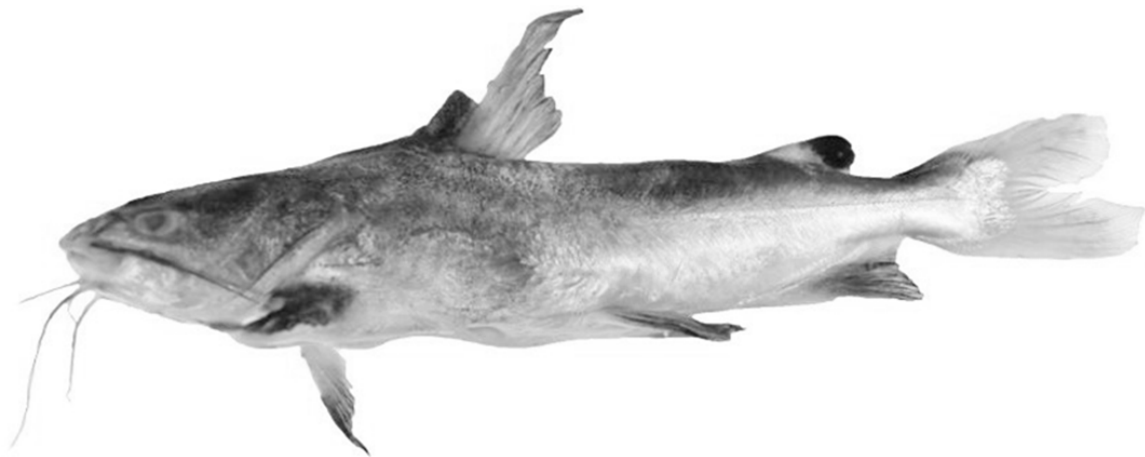


Figure 4. *Arius macronotacanthus* IPMB-I 210916-04.01, 168 mm SL, female, Kota Marudu fish market, 21 September 2016 (not to scale).

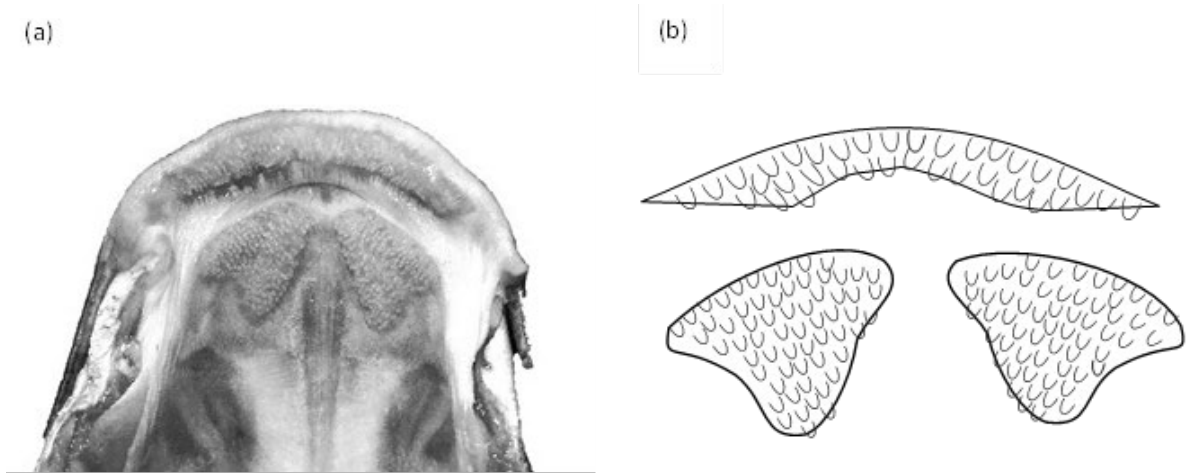


Figure 5. An image (a) and an illustration (b) of the palatal tooth patches of *Arius macronotacanthus* IPMB-I 210916-04.01, 168 mm SL, female, Kota Marudu fish market, 21 September 2016 (not to scale).

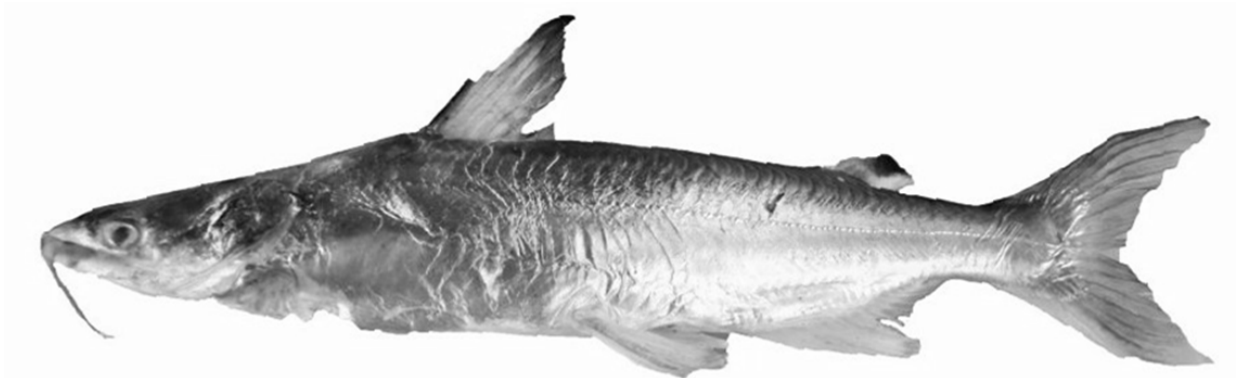


Figure 6. *Osteogeneiosus militaris* IPMB-I 250816-08.07, 261 mm SL, male, Beluran fish market, 25 August 2016 (not to scale).

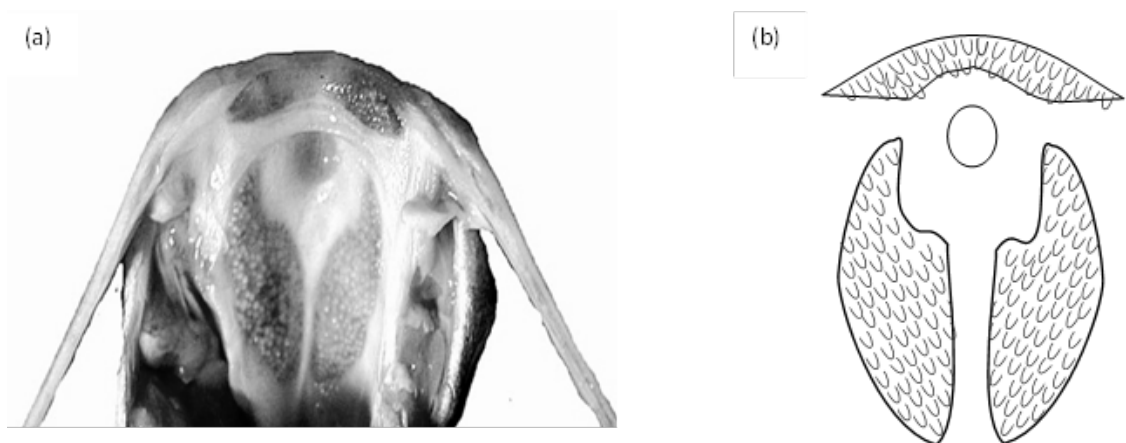


Figure 7. An image (a) and an illustration (b) of the palatal tooth patches of *Osteogeneiosus militaris* IPMB-I 250816-08.07, 261 mm SL, male, Beluran fish market, 25 August 2016 (not to scale).

Pseudosciades sona has longer head, HL (27.59%–31.85% of SL), snout length, SL (28.89%–35.86% of HL), and mouth width, MW (58.04%–65.72% of HL) with smaller eye diameter (12.90%–15.75% of HL) compared to *A. macronotacanthus* and *O. militaris*. The distance between below the eye to the bottom of the head, EH (18.95%–24.57% of HL) in *P. sona* is also longer compared to *A. macronotacanthus* (14.34%–20.94% of HL) and *O. militaris* (7.32%–13.73% of HL). Additionally, this species has longer pectoral-fin base, DL (5.21%–6.18% of SL) compared to *A. macronotacanthus* (2.98%–5.38% of SL) and *O. militaris* (3.80%–5.17% of SL).

The IUCN Red List (2024) currently lists the assessment status of 31 Ariidae species found distributed in the Western Central Pacific, but excluding *P. sona*. Most of the species are listed as least concern (LC) status, with one species listed for each of the following categories: critically endangered (CR), endangered (EN) and vulnerable (VU) species status.

In Malaysia and the Southeast Asian region, catfishes have long been referred to as a “poor man’s fish”, where because of “myths” surrounding the way these fishes obtain their food as scavengers, render the prices of this group of fishes as inherently lower (RM7-8 per kilograms) compared to other kinds of marine fishes of similar size (upwards of RM10 per kilograms) (based on survey and interview data of the present study). This relatively cheaper fish is nevertheless, a commercially important fisheries resource. As reported in the “Economics of Fisheries and Aquaculture in The Coral Triangle (CT)” (Asian Development Bank, 2014; Ahmad et al., 2016), sea catfishes are a particularly popular group of fish, and is one of the highly consumed fish in the CT countries. Moreover, in Malaysia, recent annual fisheries landing statistics indicates that marine catfishes are landed throughout the year, with 24,308 and 18,341 metric tonnes of ariids caught in 2019 and 2020, respectively (DoFM, 2019, 2020). As such, while Ariidae continue to be exploited as human food fish, and at the same time new species are continually being recorded (this study), thus, it is important to monitor the catfish species for sustainable fisheries resource management.

Comparative material examined

7 specimens *Arius macronotacanthus*: 2 specimens (IPMB-I 010716-17.05 180 mm SL; IPMB-I 010716-17.06, 188 mm SL), 1°50.97' N, 102° 51.33' E, Parit Besar fish landing site, Batu Pahat, Johor, 0-100 m depth, trawl, 1 July 2016; and 5 specimens (IPMB-I 210916-04.01, 168 mm SL; IPMB-I 210916-04.02, 162 mm SL; IPMB-I 210916-04.03, 143 mm SL; IPMB-I 210916-04.04, 142 mm SL; IPMB-I 210916-04.05, 132 mm SL), 6° 29.97' N, 116° 46.24' E, Kota Marudu market, trawl, 21 September 2016. 10 specimens *Osteogeneiosus militaris*: 2 specimens (IPMB-I 010716-17.12, 178 mm SL; IPMB-I 150716-17.11, 246 mm SL), 1° 51.10' N, 102° 51.30' E, Parit Besar fish landing site, Batu Pahat, Johor, 0-100 m depth, trawl, 15 July 2016; 4 specimens (IPMB-I 120816-17.01, 274 mm SL; IPMB-I 120816-17.02, 258 mm SL; IPMB-I 120816-17.03, 255 mm SL; IPMB-I 120816-17.04, 260 mm SL), 1° 50.47' N, 102° 52.94' E, Parit Haji Salam fish landing site, Batu Pahat, Johor, 0-100 m depth, trawl, 15 July 2016; 1 specimen (IPMB-I 250816-08.07), 5° 50.35' N, 118° 7.26' E, Sandakan wet market, Sabah, 0-100 m depth trawl; 3 specimens (IPMB-I 151016-80.04, IPMB-I 151016-80.05, IPMB-I 151016-80.06), 1° 40.70' N, 110° 25.56' E, Sungai Bako, Kuching, Sarawak, fish net; as well as *Hemiarus verrucosus* (n=7) and *Hemiarus stormii* (n=4) (Ng, 2003).

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Cinnamon Essential Oil (EOCIN) Functional Diet: Effect on Growth Performance and Health Status of *Penaeus vannamei* in Super-Intensive Tank Culture

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Abstract

A field trial was conducted to assess the application of cinnamon essential oil (EOCIN) in the super-intensive culture of white shrimp (*Penaeus vannamei*). The objective of this study is to determine the efficacy of EOCIN on growth performance and prophylaxis in prevention against two common diseases in farmed shrimps, specifically acute hepatopancreatic necrosis disease (AHPND) and *Enterocytozoon hepatopenaei* (EHP). The EOCIN dose regime, set at 1.5% (v/w kg feed), was administered for 14 days in two series of application. The experiment was carried out in two replicates for the EOCIN and control involving four (4) tank cultures, 34 m in diameter with estimation of 300,000 pieces of shrimps per tank at stocking density of 300 pieces/m² in a single cycle of culture production. An increased in weight gain of shrimp from EOCIN group was recorded compared to control, particularly in grow-out shrimps at DOC40-56 after completion of 28-days of EOCIN application. Shrimp Specific Growth Rate (SGR) showed a compensatory growth of 4.5856% day⁻¹ from the EOCIN group compared with control, 4.0586% day⁻¹. Average daily weight gain for EOCIN (0.2328 g/day) was higher than control (0.1733 g/day), with survival rates of 80% and 75%, respectively. The FCR for EOCIN was lower (1.503) compared to control (2.014). The EOCIN additive regime used in this study improved the growth performance of white shrimps in particular, prevention against AHPND and control low prevalence of EHP infection detected in the early culture.

Keywords: EOCIN, feed additive, growth, health, white shrimp

Introduction

For decades, humans rely on natural ingredients from herbal and plants for remedy in treatment of various health problems in humans and animals. Commercial essential oils such as cinnamon, garlic, lemongrass, lime and lemon are well-known with their broad range of

antibacterial, antiviral and antifungal properties and as such can be utilised as antimicrobial agents (Tariq et al., 2019; Purkait et al., 2020). Essential oils have also been shown to inhibit the growth of drug-resistant microbial strains which makes it even more challenging to be treated by conventional antibiotics (Tariq et al., 2019). Essential oils are regarded as GRAS (generally regarded as safe) grade chemicals by the U.S. Food and Drug Administration (FDA), hence, continuous treatment as preventive measures or supplement reduces the likelihood of resistance towards drug/biochemical and less side effects of toxicity issues while utilizing organic compound for a long period of time.

Research and development studies on antimicrobial effects of herbal/plant extracts and their uses (benefits and treatment) to cultured aquatic animals are still at the infant stage. Currently, few commercial essential oils known to contain antimicrobial properties have been used as feed additive and appetite enhancer to improve growth performance and prevent disease outbreak in fish and shrimp culture (Burt, 2004; Ghafoor, 2020; Bandeira Junior et al., 2022). Study has shown that cinnamon and clove oil did not show any cytotoxic effect at dosage level from 1000 to 2000 $\mu\text{g mL}^{-1}$ (Purkait et al., 2020). Such results evince that cinnamon and clove oils, used separately, or in combination, are a potential source of safe and effective natural antibacterial, antifungal and antioxidant blend in animal-feed and other pharmaceutical products (Purkait et al., 2020).

The *in vitro* antibacterial effect of essential oil has been reported against bacteria isolated from fish, such as *Citrobacter freundii* and *Aeromonas hydrophila* (Öntaş et al., 2016; Snuossi et al., 2016; Majolo et al., 2017). Supplementation of 1 mL of cinnamaldehyde per kg of diet in Nile tilapia fingerlings improved the feed conversion ratio (FCR) (days 46–60) and increased body weight gain and feed intake (days 61–75) (Amer et al., 2018). In another study, an improvement in growth performance was reported in white shrimps (*P. vannamei*) culture supplemented with plant derived essential oils blend in diet and a lower mortality rate when challenged against *Vibrio parahaemolyticus* bacteria causing acute hepatopancreatic necrosis disease (AHPND) compared to the positive control (unsupplemented) which received no treatment (Hoa et al., 2023). The *Vibrio* spp. count in the hepatopancreas of shrimps fed with 2 g kg^{-1} of the essential oil blend was marked lower compared to that of the control.

In the aquaculture industry, cinnamon (*Cinnamomum zeylanicum*), is one of the oldest medicinal plants widely used globally, where it is used in the form of powder or essential oil (El-Hack et al., 2020). Essential oil extracts possess good antimicrobial effects against *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Staphylococcus aureus* (Purkait et al., 2020). In another report, it is reported that essential oils are effective against *V. parahaemolyticus*, *Escherichia coli*, *Staphylococcus epidermidis* and *Enterococcus faecalis* (Chang et al., 2001) as well as promoting the stimulating effect on the digestive system (Ertas et al.,

2005). Hence, these herbs and their extracts received more attention as growth promoter alternatives.

AHPND in Malaysia was first detected in 2011 and has been linked with high mortalities of cultured white shrimps (*P. vannamei*). Following the outbreaks from 2011 to 2014, a disease surveillance program was implemented by the Department of Fisheries Malaysia (DOF) with active involvement of farmers and other stakeholders to monitor and control disease outbreaks in shrimp farms. A study on the status of AHPND in 2019/2020 showed that State of Pahang, among the major producers of white shrimp culture, had the highest prevalence of AHPND (23%), followed by Perak (11%), Sarawak (7%) and Kedah (1%) (Padilah et al., 2022). The disease is known to be endemic in nature due to the presence of disease agent *V. parahaemolyticus* and other *Vibrio* spp. carrying virulent plasmid and *PirA/B* toxin genes in the aquatic environment. Presently, AHPND has been under control through management approach of creating healthy shrimps fed with vitamins and/or other health supplement diet, as well as good management of water quality in the pond culture.

Countries in Southeast Asia such as China, Vietnam, Thailand and Malaysia have suffered from *Enterocytozoon hepatopenaei* (EHP) outbreaks during the last decade and eventually, have also had AHPND outbreaks every now and then (Aranguren et al., 2017). EHP causes growth retardation and increased size variability, and in more advanced stages, the infected shrimp have soft shells and exhibit lethargy, reduced feeding and empty midguts. It is possible that EHP favours the establishment of AHPND and other bacterial diseases, such as Shrimp Hepatopancreatic Necrosis (SHPN) and White Faeces Syndrome (WFS). The strong association was found between cases of SHPN and EHP which suggests that EHP increases shrimp susceptibility to infection by *Vibrio* spp. (Aranguren et al., 2017). EHP infection disrupts the cells of the hepatopancreas tubules and allows *Vibrio* spp. to colonize the sloughed cells and the exposed basement membrane leading to the state of illness or infections (Aranguren et al., 2017).

In the late 2000s, shrimp farmers implemented more intensive practices known as super-intensive farming technologies, driven mainly by the search for greater shrimp farm productivity. The super-intensive system for white shrimp, *P. vannamei*, as one that uses lined ponds, raceways, or tanks for stocking densities of over 150 shrimp per m² during growth and applies a significant level of technology. While commercial intensive shrimp systems in Asia have shown good results (Taw, 2017), the commercial economic viability of super-intensive systems has not yet been proven. The challenges in intensive shrimp systems include prevalence of diseases, feed efficiency, energy use, and genetic selection in terms of the organism and environmental, social, and welfare issues for the farmers (Villarreal & Juarez, 2022). Diseases were responsible for a significant shrimp production decline from 2009 to

2014, when around 1 million tons were lost, mainly in Asia and Latin America. White spot syndrome virus (WSSV/WSD), AHPND, EHP, and WFS, have impacted the industry with substantial economic losses (Shinn et al., [2018](#)), so that producers look for more controlled intensive systems with higher biosecurity in an effort to mitigate this risk.

Feeding shrimps with functional diets such as mixed essential oils and herbs can be an advantageous strategy to mitigate infectious diseases, consequently, reduce the rise of antibiotic resistance problems of pathogenic bacteria towards treatment and build an environmentally friendly and sustainable shrimp aquaculture. Diet supplemented with a mixture of essential oils from thyme and cinnamon significantly improved the resistance of *P. vannamei* towards AHPND (Cabanyero et al., 2023). Survival of shrimps challenged with *V. parahaemolyticus* increased when they were fed with essential oils-enriched diet for 4 to 5 weeks. This feeding strategy also reduces the percentage of 'carrier' after induced infection or in the stressful situation (Cabanyero et al., 2023).

A field trial on oral diet of EOCIN in *P. vannamei* culture was carried out to determine the efficacy of EOCIN dose-regime used on the growth performance and health status of white shrimp culture in super-intensive tank system, in particular against AHPND and EHP.

Materials and Methods

Field trial and sampling size

White shrimps were cultured at stocking density of 350 pieces per m² in 4 large round PVC tank, 34 meter wide with capacity of 1000 tonnes of water and estimation of 300,000 pieces of post larvae (PL-10) per tank at initial stocking. The study was carried out in two replicates for control (2) and EOCIN (2). The control groups were fed with a normal commercial based diet whereas the EOCIN group were fed with EOCIN mixed in feed pellet at 1.5% v/w kg of feed (3% body weight) for 14 days in two series of application at the age day of culture (DOC)1-14 and DOC40-54. A total of 210 white shrimps were sampled from the post larvae shrimps (PL-10) before stocking (n=10), the control (n=100) and EOCIN (n=100) with 6 times of sampling throughout the 56 days of culture. The second sampling was carried out after completion of the 14 days of EOCIN application from EOCIN group (2 tanks, n=20) and control (2 tanks, n=20) at day of culture-16 (DOC16, total, n=40), followed by sampling of shrimps before the second application of EOCIN started at DOC40 (n=40), during treatment, DOC45 (n=40), DOC47 (n=40) and DOC56 (n=40) after completion of treatment. Sampling of shrimps were carried out in the morning before feeding. Sample of hepatopancreas tissues was fixed in absolute alcohol for PCR analysis of AHPND and EHP, whereas the presence of bacteria in the hepatopancreas organs of shrimp was isolated using direct inoculation method on the culture media.

EOCIN treatment regime and preparation in feed pellet

The feeding regime of EOCIN was given at 1.5% v/w kg feed (3% BW) for 28 days in two series of applications of 14 days regime with 25 days interval between them. The first feeding of EOCIN was given for 14 days continuously right after the admission of post larvae shrimps into the super-intensive tank culture (DOC1-14) followed by the second regime of treatment at DOC40-54. Around 1.5 mL of EOCIN was mixed in 50 mL of sterile water and sprayed evenly onto 15 kg of commercial based feed pellet (prawn feed grower) before feeding to the shrimps. The control shrimps were fed with a commercial pellet-based diet throughout the study and the EOCIN group shrimps were fed with EOCIN mixed pellet from DOC1-14 and DOC40-54 whereas for other days, they were fed with normal commercial based diet. The shrimp was fed four times per day at 7-7:30 am, 10:30-11:00 am, 1:30-2:00 pm and 5:00-5:30 pm. The composition of a commercial prawn feed grower pellet used in the study is shown in Table 1.

Table 1. The composition of commercial prawn feed grower pellet.

No.	Composition of grower pellet	Percent (%)
1.	Crude Protein (min)	40-44%
2.	Lipid (fat)	6%
3.	Moisture (max)	12%
4.	Crude fibre (max)	3%
5.	Ash (max)	15%

CTAB-DTAB DNA extraction protocols

A method described in the manual for IQ2000™ AHPND/EMS Toxin 1 was adhered to (GeneReach Biotech. Corp. Taiwan). Briefly, sampled of 10 PLs and hepatopancreas tissue of shrimps from DOC16 onward, weighing about 15-30 mg in the 1.5 mL tube were grinded until all tissues are thoroughly mixed and dissolved into 600 µl DTAB solution. The tissues in a tube was incubated at 75 °C for 5 minutes, which was proceeded to cool down to room temperature, vortexed briefly, then added with 700 µl of chloroform. It was then vortexed again, with the mixture centrifuged at 12,000 x g for 6 minutes. About 200 µl of the upper aqueous phase was transferred into a new tube, then added with 100 µl CTAB solution and 900 µl deionized water, vortex briefly and incubated at 75 °C for 5 minutes. The sample was cooled down to room temperature, then centrifuged at 12,000 x g for 10 minutes. The supernatant was discarded, the pellet was re-resuspended with 150 µl dissolving solution, followed by incubation at 75 °C for 15 minutes, cooled down to room temperature and centrifuged at 12, 000 x g for 5 minutes. The clear solution was transferred into a new tube containing 300 µl of 95% ethanol, vortex briefly and centrifuged at 12,000 x g for 5 minutes. The supernatant was discarded, the pellet was washed with 200 µl of 75% ethanol, centrifuged at 12,000

x g for 3 minutes. After the disposal of the supernatant, the DNA pellet was dried and dissolved in 100-200 µl of deionized water or TE buffer. The DNA sample was stored at -20 °C until use for further analysis.

Polymerase chain reaction (PCR): Reagent and amplification protocol for AHPND

Pre-mix reagent (12.5 µl) and IQzyme DNA Polymerase (2U/µl; 0.5 µl) was prepared for a total of 13 µl/reaction per test. Samples was run with 2 positive controls (10^3 and 10^2) and 1 template control (NTC; deionized water or yeast tRNA). The reaction condition for thermocycler was set as the following: 94 °C 20 sec; annealing 62 °C 20 sec; 72 °C 30 sec, repeat 35 cycles, 72 °C 30 sec final extension; 20 °C 30 sec at the end of the final cycle.

PCR EHP

Method described by Jaroenlak et al. (2016) was used for detection of spore wall protein gene-specific for EHP (SWP-PCR). The primer sequence for EHP was: First PCR: 1F-TTGCAGAGTGTTAAGGGTTT; 1R-CACGATGTGTCTTTGCAATTTTC; Nested PCR primer; 2F-GCTGTTTGTCTCCAAGTATTTGA; 2R-TGGCGGCACAATTCTCAAACA and the amplification protocol for EHP was according to the following: **First PCR**; 94 °C 2 min; 94 °C 20 sec, annealing 60.2 °C 20 sec; 72 °C 30 sec; repeat 35 cycles; Final extension 72°C 4 min; **Nested PCR**: 94 °C 2 min, annealing 58.3 °C 20 sec, 72 °C 30 sec; final extension 72 °C 4 min; 20 °C 30 sec at the end of the final cycle.

Electrophoresis, gel staining and image documentation

A 2% agarose gel was prepared by adding 2 g of agarose gel with 100 ml TBE buffer in a wide mouth glass bottle. The mixture is then heated in the microwave oven for 1.5 to 2 minutes until it becomes hyaline which consequently leads to its cooling to 50 °C before pouring into the gel cast with the plastic comb and blockers. For electrophoresis, 5 µl of PCR product-loading dye mixture was loaded into each well with DNA molecular weight marker serve as a reference for PCR product size. Electrophoresis was run at a constant voltage of 100 V for 30 minutes and viewed under Bio-imaging system (Syngene, Cambridge, UK). For AHPND, negative sample showed only the 712 bp band (internal control) and AHPND plasmid negative whereas a band at 218 bp and 432 bp indicate a positive sample with toxin 1 and virulent AHPND plasmid. A band at 432 bp only (with or without 712 bp band) indicates AHPND plasmid with toxin 1 deletion, non-virulent. For EHP, positive sample was categorized into heavy and light infections based on detection of amplicon at 514 bp (heavy) or 148 bp (light).

Bacteria culture and isolation

Direct inoculation method was used for isolation of bacteria from the hepatopancreas organ. Biochemical analysis for identification of isolates was done following the method described by Austin & Austin (1999). Pure isolate from tryptone soya agar (TSA+ 1.5% NaCl) plate was identified by routine testing using Gram staining, oxidase, catalase, sulfur, indole, motility test (SIM) and the triple sugar iron (TSI) test. Colony morphology of *Vibrio* spp. was determined from pure isolate culture of bacteria on thiosulfate citrate bile salt agar (TCBS) and biochemical test for confirmation to genus and species using API 20NE (BioMérieux, Marcy l'Etoile, France).

Calculation of growth parameters

To assess the growth performance of the shrimp in the feeding experiment, the following formulas are used:

- a) Daily weight gain (DWG, g day⁻¹) = (W_f – W_i) / t (rearing period, day)

$$DWG = \frac{W_f - W_i}{t} \quad \text{(Equation 1)}$$

where,

W_i = initial average weight (g)

W_f = final average weight (g)

t = rearing period (day)

- b) Specific growth rate (SGR, % day⁻¹) = [Ln(W_f) – Ln(W_i)] * 100/t (rearing period, day)

where,

W_f = the final average weight (g)

W_i = the initial average weight (g) of shrimps during the rearing period (day).

t = rearing period (day)

- c) Survival Rate (SR, %) = (final population of shrimp at harvest/initial population of post larvae stocked) x 100. The SR of *P. vannamei* shrimp is the survival rate during one cultivation cycle.

$$SR = \frac{N_t}{N_o} \times 100 \quad \text{(Equation 2)}$$

where,

SR = Survival rate (%)

N_t = Final population (individual)

N_o – Initial population (individual)

d) Feed conversion ratio (FCR) = feed given/fish weight gain

Feed conversion ratio (FCR) is a unit to describe a certain feed amount to earn one kilogram of biomass. FCR was calculated using the formula below:

$$FCR = \frac{F}{[(B_t + B_m) - B_o]} \quad \text{(Equation 3)}$$

where,

FCR = Feed conversion ratio

F = Total feed amount (g)

B_t = Final biomass (g)

B_m = Deceased biomass (g)

B_o = Initial biomass (g)

Calculation of bacterial prevalence (%) and statistical analysis

The prevalence of bacteria identified from the white shrimps were calculated as mean prevalence percentage (%). The prevalence (P) was estimated by dividing the number of samples that tested positive (S_i) for the specific bacterial divide by the total number of samples x 100 from each sampling group (S_N), $P = S_i / S_N \times 100$. Descriptive statistics, independent t-test was used to describe the results of bacterial (n) isolated from EOCIN and control. Body weight gain was analysed as measurement on growth performance using IBM SPSS Statistics Version 25, (2017) for significant difference ($p \leq 0.05$) between the control and EOCIN fed group.

Results and Discussion

Growth performance and body weight measurement

Higher daily weight gain was recorded in white shrimps from the EOCIN group compared with the control from DOC40-56. The EOCIN treated shrimps had higher average final body weight gain (BW = 13.09 ± 2.20) than the control (BW = 9.75 ± 1.99), however, statistical analysis using independent Mann-Whitney U test showed no significant difference ($p=0.280$, $p>0.05$) in body weight gain between the two groups after 56 days of culture. The difference in average body weight gain between EOCIN and control are shown in Figure 1. Higher body weight gain was recorded from DOC40-56 from shrimps that received EOCIN diet, but the overall growth performance did not reach the optimum potential due to light infection of EHP that was detected in the post-larvae shrimps (PL10, 10%) and white shrimps from EOCIN group at day-16 of the culture (DOC16, 20%). Results of growth performance analysis of white shrimps between EOCIN treated group dan control are

shown in Table 2. EHP infection is known to cause slow growth and increase variation in size.

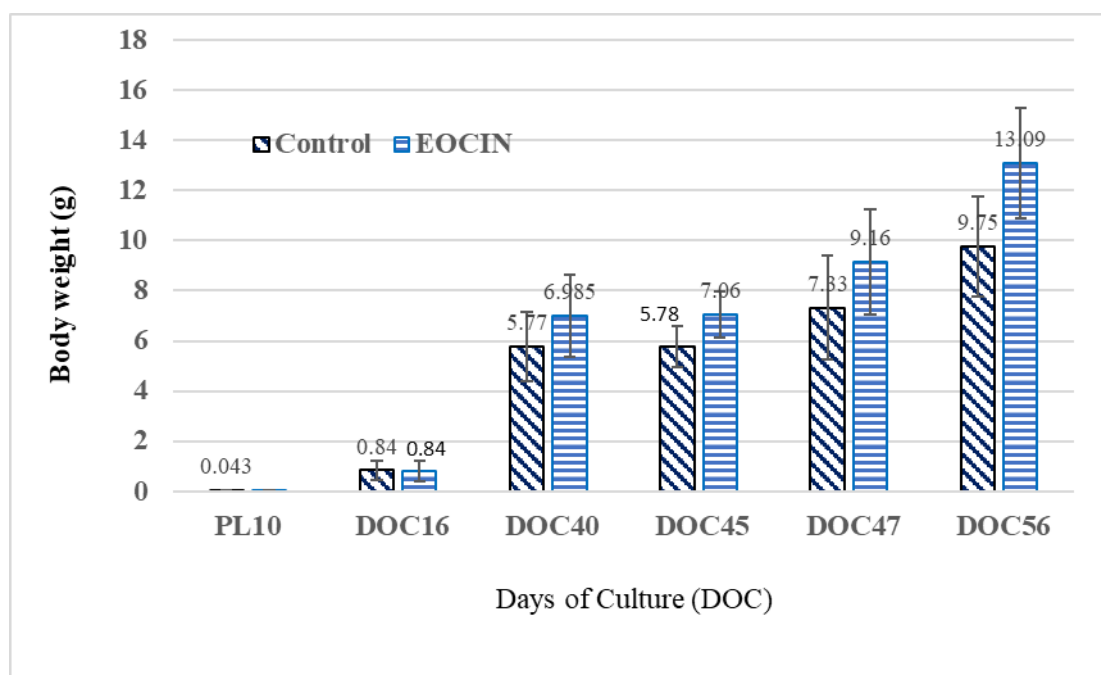


Figure 1. Comparison of body weight gain of *P. vannamei* culture between EOCIN treated group and control.

After completion of EOCIN treatment, The SGR values showed that the compensatory growth does occur in shrimps treated with EOCIN ($4.5856\% \text{ day}^{-1}$) compared to control ($4.0586\% \text{ day}^{-1}$) (Table 3). Average daily weight gain (DWG) for EOCIN (0.2328 g/day) were also higher than the control (0.1733 g/day). Shrimps treated with EOCIN showed higher feed efficiency (FCR, 1.503) although it is common to obtain the FCR values of 1.5 to 2.5 in *P. vannamei* culture. EHP infection was suspected by farmers when FCR was higher and DWG of cultured *P. vannamei* shrimp was below 0.35 g day^{-1} . Normal average daily gain for shrimp would be about 0.25g up to 0.3g/per day (Hoa et al., 2023). The control group had higher FCR (2.014), showed by lower body weight gain and increase variation in size (Figure 1) suspected due to EHP (infected PL) although the control shrimps was tested negative at DOC16-56.

FCR values vary with the production system and the type of feed used. FCR of 1.4-1.8 are generally obtained from intensive whiteleg shrimp farming and FCR of 1.8 is used by FAO Fisheries and Aquaculture department to compare with the existing efficient management (Briggs, 2006). FCR as low as 1.2 can be achieved when applying a superior quality of feed, yet many shrimp farmers experience FCR of higher than 2.2 (Chaikaew et al., 2019; ASEAN, 1998). Low FCR values can be ascribed by various reasons, such as the strict control of feeding in closed system (Dien et al., 2018), partial harvest and shorter period of culture for reaching harvest size (Thakur & Lin, 2003), and seasonal variation (Teichert-Coddington, Martinez & Ramirez, 2000). On the contrary, Lim (2010)

listed five common mistakes that lead to the high FCR: usual high-water temperature, overfeeding, over frequent feeding, fast water current from aerators, and insufficient aeration. A high FCR of 2.0 in the control group indicates that this farm requires better feed management strategy such as EOCIN supplement regime in diet to enhance shrimp appetite and their growth performance.

Table 2. Growth performance analysis of white shrimps between EOCIN treated group and control.

Day of culture (DOC)	DOC1-56	
Group	Control	EOCIN
1. Mean Initial weight	0.043±0.014	0.043± 0.017
2. Mean Final weight	9.75±1.99	13.09±2.20
3. Average daily weight gain (DWG)	0.1733	0.2328
4. Specific growth rate (SGR% day ⁻¹)	4.0586	4.5856
5. Survival rate (SR%)	75	80
6. Feed conversion ratio (FCR)	2.014	1.503

Table 3. The prevalence of AHPND and EHP in *P. vannamei* between control and EOCIN.

Trial 1	No of samples	Age	EOCIN		Control	
			AHPND	EHP	AHPND	EHP
1	10	PL10	0(0/10)	10(1/10)	0(0/10)	10(1/10)
2	40	DOC16	0(0/20)	20 (4/20)	0(0/20)	0(0/20)
3	40	DOC40	0(0/20)	0(0/20)	0(0/20)	0(0/20)
4	40	DOC45	0(0/20)	0(0/20)	0(0/20)	0(0/20)
5	40	DOC47	0(0/20)	0(0/20)	0(0/20)	0(0/20)
6	40	DOC56	0(0/20)	0(0/20)	0(0/20)	0(0/20)
Total	210	Prevalence (%)	0%	10-20%	0	10%

FCR values vary with the production system and the type of feed used. FCR of 1.4-1.8 are generally obtained from intensive whiteleg shrimp farming and FCR of 1.8 is used by FAO Fisheries and Aquaculture department to compare with the existing efficient management (Briggs, 2006). FCR as low as 1.2 can be achieved when applying a superior quality of feed, yet many shrimp farmers experience FCR of higher than 2.2 (Chaikaew et al., 2019; ASEAN, 1998). Low FCR values can be ascribed by various reasons, such as the strict control of feeding in closed system (Dien et al., 2018), partial harvest and shorter period of culture for reaching harvest size (Thakur & Lin, 2003), and seasonal variation (Teichert-Coddington, Martinez & Ramirez, 2000). On the contrary, Lim (2010) listed five common mistakes that lead to the high FCR: usual high-water temperature, overfeeding, over frequent feeding, fast water current from aerators, and insufficient aeration. A high FCR of 2.0 in the control group indicates that this farm requires better feed management strategy such as EOCIN supplement regime in diet to enhance shrimp appetite and their growth performance.

Bacterial prevalence (%)

Gram negative bacteria were commonly isolated from hepatopancreas of white shrimps with highest prevalence in the control group consisting of *V. parahaemolyticus* (35-60%), *V. vulnificus* (40-60%) and *A. hydrophila/caviae* (20-26.7%). *V. parahaemolyticus* (15-20%) and *A. hydrophila* (10-15%) were isolated at much lower prevalence following EOCIN treatment at Day-16, Day-47 and Day-56 of culture. Although prevalence of *V. vulnificus* (10-13.3%) was much lower in EOCIN group, the result was inconsistent throughout the study since a high prevalence was detected at DOC56 (60%) after completion of 28 days of treatment (Figure 2). Other bacteria isolated were *Pasteurella multocida* (6.7-20%) and *Mannheimia haemolytica* (5%) with low prevalence.

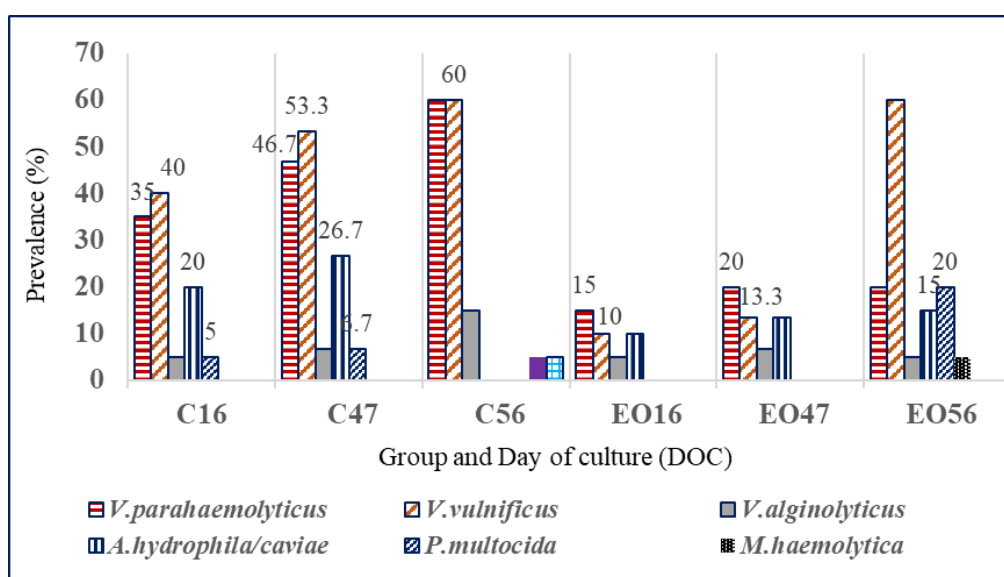


Figure 2. Bacterial prevalence from white shrimps in control (C) and EOCIN (EO) at different day of culture (DOC).

Analysis for the Levene test showed that $p \geq 0.05$ ($p=0.796$), whereby the variance (*V. parahaemolyticus*) is not significantly different from each other in group, hence the homogeneity of the variance is met. Independent sample test showed the p-value of 0.03 ($p \leq 0.05$) indicating that there was a significant difference in *V. parahaemolyticus* prevalence isolated from EOCIN and control shrimps. Statistical analysis in the prevalence of other bacteria (*V. vulnificus*, *V. alginolyticus* and *A. hydrophila*) isolated from shrimps following EOCIN treatment showed no significant difference ($p \geq 0.05$) from the control.

In this study we determine the microbial isolated from the hepatopancreas of white shrimps to see their connections with the disease incidence, in particular AHPND. Estimation of bacterial prevalence is fundamental in determination and management of population health, disease dynamics and infections risk factors. It is known that members of *Vibrionaceae* are the natural pathogens of the aquatic marine environment. Microbiota of shrimp's digestive system which includes the stomach, hepatopancreas and intestine are closely related to the environmental condition, as well as host developmental stage and health status of shrimp culture (Cheung et al., 2015; Cornejo-Granados et al., 2018). High prevalence of *V. parahaemolyticus* opportunistic pathogen in the gut microbiota will increase the risk of the shrimps to infection following the colonization of bacteria on the epithelial cell walls. Thus, presence of *V. parahaemolyticus* in the hepatopancreas organ pose a high risk for the microbe to invade hepatopancreas cells under any stressful conditions. *Vibrio* spp., in particular *V. parahaemolyticus* pathogen carrying virulent factor 'Plasmid' and *PirA/B* toxin can cause AHPND which has a possibility of resulting in high mortality rates of susceptible shrimp aged below DOC30 (Lightner et al., 2012). EOCIN treatment regime at early culture (DOC1-14) intended to boost the immune function, prevent stress and reduce the prevalence of *V. parahaemolyticus*, hence, reduce the risk of AHPND whereas EOCIN treatment at juvenile/grow-out stage (DOC40-56) help to boost the growth performance.

The effectiveness of cinnamon essential oil as antimicrobial agent was studied by many researchers. Antimicrobial activities of EOCIN inhibit *Vibrio* spp. from colonizing in the hepatopancreas organ of white shrimp. Our study showed that EOCIN treatment regime used helps to control AHPND and EHP in the early stage of culture and juvenile shrimp. Minimum inhibitory concentration of EOCIN at $1400 \mu\text{g mL}^{-1}$ and minimum bactericidal concentration (MBC) at $1500 \mu\text{g mL}^{-1}$ able to suppress the biofilm formation and swarming motility of *V. parahaemolyticus*, thus indirectly affecting the virulence activities of this bacteria (Domínguez-Borbor et al., 2020).

Conclusion

EOCIN has a promising use in aquaculture. A holistic approach is required in relativity in evaluating the potential use of EOCIN as functional diet for shrimp, particularly towards the control of *V. parahaemolyticus* AHPND bacterium and microsporidia protozoa EHP. EOCIN regime in two

applications at 1.5% (v/w kg feed) for 14–28 days are proven effective to promote growth and improve health status of white shrimp culture. EOCIN improves the growth performance of white shrimps as shown by an increase in DWG and SGR %/day, increase survival rate and reduced FCR. The management approach during culture by using alternative therapeutics, such as EOCIN, coupled with improvements in water quality and regular fish health monitoring program may bring about reductions in bacteria and EHP in the aquatic culture system. EHP is the main problem encountered in many shrimp farms today. Disinfection of the tank culture or pond bottom treatment prior to culture can effectively kill all the spores of EHP and pathogenic bacteria. Low prevalence of EHP in the early stages of culture was managed with EOCIN treatment and improved farm management by practicing appropriate stocking density, partial harvest of culture, adoption and maintenance of hygienic practices, and implementing strict biosecurity measures to control the spread an infection. EOCIN treatment is proven to reduce the prevalence of *Vibrio* sp., particularly the colonization of *V. parahaemolyticus* in shrimp hepatopancreas tissues. Field trials carried out showed that EOCIN treatment regimens helped control AHPND and EHP in the post-larvae and juvenile stages if treated while at low prevalence.

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Appendices

Annex 1

Calculation of FCR

- 1) Control, FCR, 1: 2.014
Feed per day: 60.59 kg, Total carrying capacity: 2.654kg
Total feed: 4544.9 kg
Total product: 2255.9 kg
Partial harvest at DOC79 (76 pcs/kg), 691.9 kg;
DOC80 (76 pcs/kg), 1032 kg and DOC87 (70pcs/kg), 532 kg.
- 2) EOCIN, FCR, 1: 1.503
Feed per day: 48.5 kg, Total carrying capacity: 4.74kg
Total feed: 4273.9 kg
Total product: 2844 kg
Partial harvest DOC76 (70 pcs/kg); 770 kg
DOC78 (68 pcs/kg); 1042 kg and DOC88 (51 pcs/kg); 1032 kg

Annex 2

Levene’s Descriptive statistics for *V. parahaemolyticus*

Parameter	EOCIN	Control
Mean	2.3333	4.3333
Standard Deviation	1.2111	1.5055
Standard Error	0.4944	0.6146
N	6	6

Independent Sample test

Levene’s test for equality of variances					t-test for equality of means				
<i>V. parahaemolyticus</i>	F	Sig	t	df	Sig (2-tailed)	Mean difference	Standard error difference	95% confidence interval of the difference	
Equal variances assumed	0.070	0.796	2.535	10	0.030	-2.000	0.7888	-3.7576	-0.2424
Equal variances not assumed			2.535	9.561	0.031	-2.000	0.7888	-3.7686	-0.2314

The Potential of Ocean Thermal Energy Conversion (OTEC) in Sabah, Malaysia: A Systematic Review

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Abstract

Ocean Thermal Energy Conversion (OTEC) is a relatively new technology that leverages on the temperature difference between warm surface seawater and cold deep seawater to generate electricity with no harmful emissions. This type of energy has become an important research topic for sustainable development due to the large energy resources contained in the ocean. Malaysia has a great potential to harness this type of energy due to its long coastline. We studied Sabah's coastal waters for its potential in OTEC. Sabah's coast is blessed with a range of productive coastal marine ecosystems coupled with several deep-sea islands. These diverse marine features translate into the type of OTEC plant suitable for implementation in Sabah. A systematic literature review (SLR) approach was utilized to compile, review, and assess literature advocating for OTEC, the different types of OTEC plants and analytical methods used in OTEC plant site-selection. We identified five potential sites for OTEC plants in Sabah. These include three sites in the northeast, namely Layang-Layang Island, Mengalum Island, and Mantanani Island, and two sites in the southeast, namely Tawau and Kalumpang. The availability of potential sites in Sabah indicates that the OTEC system – not only as a source of unlimited and environmentally friendly renewable energy, but also as a means of freshwater production – is a viable option for implementation in the State. OTEC systems can be diverted to allocate a portion of the electricity generated for operating adjacent water desalination plants, for generating sample volumes of water suitable for human consumption, and aquaculture and agricultural purposes.

Keywords: Ocean Thermal Energy Conversion (OTEC) system, OTEC plant type, renewable energy, clean energy, Sabah

Introduction

The transition from conventional to clean energy sources represents one of the most promising developments in recent years for addressing climate change. It holds substantial implications related to economic policy agendas, as policymakers strive to incorporate clean energy into transforming energy systems (Özgül et al., 2020). Various renewable energy sources, such as solar, hydroelectric, wind, and nuclear energy have been proposed to meet energy demands in response to the worldwide difficulties in addressing energy issues and global warming.

The deep seas in East Malaysia (the Borneo archipelago), have a potential to generate renewable and stable energy. According to Martin et al. (2016), the deep seawater within the (Borneo) archipelago holds a promising utility as a resource for a variety of industries. Deep seawater has huge potential as a nutrient provider to the surface ocean (Takahashi, 2003), while seawater air conditioning (SWAC) can be tapped to reduce energy consumption (Hernandez et al., 2019). Such areas with access to both deep seawater and surface seawater have a huge capacity for Ocean Thermal Energy Conversion (OTEC) to supply adequate base-load electricity. The proficiency in OTEC in the State appears substantial and comparable to regional tropical and subtropical nations, such as Indonesia (Fahmie et al., 2018; Langer et al., 2021; Rahmawati et al., 2021), the Philippines (Uehara et al., 1988; Fahmie et al., 2018; Ding et al., 2022), and Nauru (Wolff et al., 1979 in Thirugnana et al., 2021).

The Sabah State Government have recently (in April 2024) approved two new legislations to pave the way for renewable energy in the State, specifically the Ocean Thermal Energy Conversion (OTEC) Enactment 2024 and Energy Commission of Sabah Enactment (Amendment No.2) 2024 (*see* ECoS, 2024). These enactments are one of the State's main initiatives under the Blue Economy policy framework (<https://blueeconomy.sabah.gov.my/>; DE, 2024a).

The surface temperature and the ground temperature beneath the Sabah Trough align with the fundamental principles of OTEC technology. Building on a feasibility study, a group of researchers explored the possibility of developing and commercializing the first Malaysian OTEC plant in Sabah (Jaafar, 2012). However, there remains a knowledge gap about which type of OTEC plant is suitable for implementation in Sabah. Fahmie et al. (2018) later suggested that for Layang-Layang Island, an oceanic atoll approximately 180 nm (300 km) northwest of Kota Kinabalu, the capital city of Sabah, OTEC plants with a fixed platform is the most suitable design. Thirugnana et al. (2021) also highlighted Layang-Layang Island and a few other sites with significant potential for OTEC system for development in Sabah, i.e. Semporna, Tawau, Kudat and Kalumpang. However, they did not specify nor suggest the type of suitable plant at each of the locations

mentioned. A related study was undertaken by Seungtaek et al. (2020), where they conducted an economic analysis of the possibility of OTEC plants in several localities Sabah. The analyses were, however, restricted to basic open- and closed- cycle models only. They attributed the exclusion of hybrid models from their analyses to insufficient data on initial investment costs, a decision that may inadvertently introduce inaccuracies into their study.

Materials and Methods

Systematic Literature Review About Potential of OTEC in Sabah, Malaysia

A systematic literature review (SLR) was utilized to portray the current progress of OTEC in Sabah. It serves as a strategy to compile, review, and gain access to literature using predefined techniques in order to mitigate bias (Brereton et al., 2007; Gurbuz & Tekinerdogan, 2018; Salleh & Tasnim, 2022). An SLR analysis involves "being methodical, comprehensive, transparent and replicable", and our SLR analysis approach followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline, following Liberati et al. (2009), Moher et al. (2010), and Mengist, et al. (2020) as the prescribed framework for the reporting process (Figure 1). Thus, the PRISMA guideline serve as the framework to carry out the systematic review, guiding the formulation of review questions and eligibility criteria, identification of relevant literature meeting the criteria, as well as the processes of data extraction, synthesis, and presentation of results to solve the review questions.

We employed the Web of Science (WOS) and Scopus databases generated by Universiti Teknologi Malaysia's (UTM) system to obtain data used in this study. The database search processes were integrated with the phrase searching functionality and the Boolean operators OR and/or AND to efficiently combine keywords. Additionally, we complemented the database search with manual searching methods (Xiao & Watson, 2019) to ensure a comprehensive collection of relevant literature. The chosen search keywords specified research articles within the database that contain the terms in their title, abstract, or keywords section. The search keyword includes the phrases "OTEC," "ocean thermal energy conversion," "OTEC type plant," "OTEC site location," and "OTEC Sabah." Articles containing the terms "OTEC site selection" and "parameter" were also considered (Table 1).

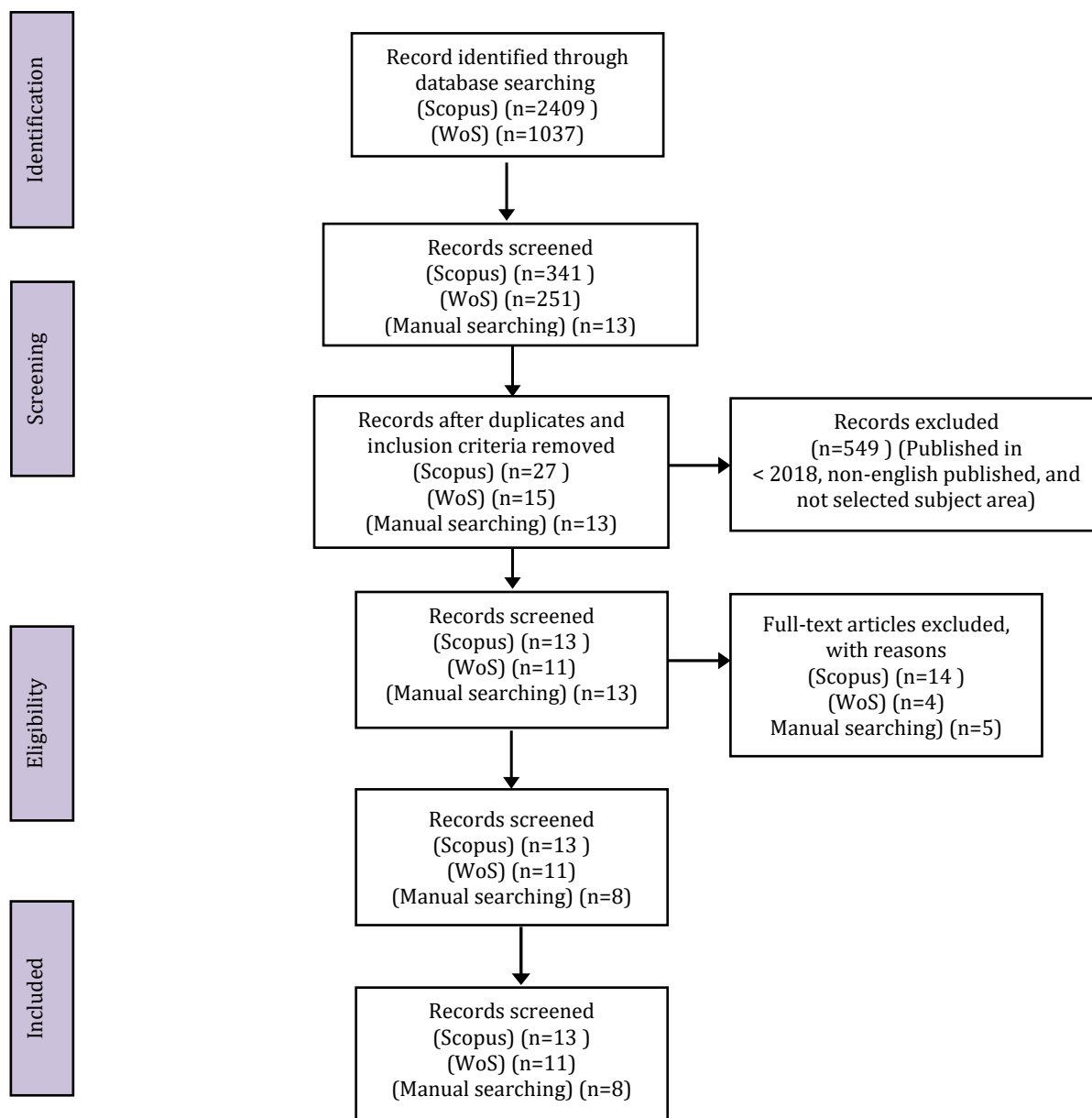


Figure 1. PRISMA flow diagram for literature search (Liberati et al., 2009; Moher et al., 2010; Mengist et al., 2020).

Table 1. Search string for Web of Science (WoS) and Scopus

Search engine	Search string
WoS	TS= ("OTEC" OR "ocean thermal energy conversion" OR "OTEC type plant" OR "OTEC site location" OR "OTEC Sabah" OR ("OTEC site selection" AND "criteria") OR ("OTEC type plant" AND "criteria") OR ("OTEC Malaysia" AND "criteria"))
Scopus	TITLE-ABS-KEY ("OTEC" OR "ocean thermal energy conversion" OR "OTEC type plant" OR "OTEC site location" OR "OTEC Sabah" OR ("OTEC site selection" AND "criteria") OR ("OTEC type plant" AND "criteria") OR ("OTEC Malaysia" AND "criteria"))

Next, a screening process was implemented, incorporating the inclusion and exclusion criteria and quality assessment. The inclusion criteria used the essential features of the target population to address research questions. In contrast, the exclusion process examines various characteristics of the population that might hinder the study or increase the risks for undesirable results that were excluded from the researcher's consideration (Patino & Ferreira, 2018). The predefined literature inclusion and exclusion criteria achieved in this study are presented in Table 2.

Table 2. The inclusion and exclusion criteria

Criterion	Inclusion	Exclusion
Timeline	Between 2018-2023	<2018
Language	English	Non-English
Subject area	OTEC-related terminology or antonyms (type, site location, site selection)	Other than OTEC-related terminology or antonyms (type, site location, site selection)
Search engine	Web of Science (WoS), Scopus	Other than Web of Science (WoS), Scopus

The conclusive inclusion process led to the selection of 32 articles for further analysis. Of these, 13 articles were obtained from Scopus, 11 from Web of Science, and the remaining eight were manually added due to fundamental importance to the possible development of OTEC in Malaysia. Those articles were categorized into five sections to enable a thorough analysis. The sections include the year of publication, journal names, journal ranking, research methods utilized, and research topics.

Results

Publication Year of Articles

The present study shows a worldwide increase in trends of publications of OTEC-related articles over the years (Figure 2). The selected articles include three articles which were published before 2018, as the crucial information in such literature are relevant in this study. This included one article each published in 2012, 2015 and 2016. There were two articles from 2019, and three from 2018, 2020, and 2023. The highest number of selected articles were published in 2021 and 2022, with a total of 6 and 12 articles respectively.

Journal Characteristics

The journal characteristics focused on the type of document, journal ranking, and source of publication. In this study, a total of 19 articles, 8 conference papers, 3 presentation slides, 1 proceeding paper and 1 book chapter were extracted from both the search engines and manual

searching process. Next, the journal ranking was determined from the quartile of each document. The ranking was derived from the citation metrics which is used to assess the quality of a scientific publication. A high-quality article from a reliable publication source was indicated by the higher quartile. Therefore, Q1 represents the most esteemed journals in the field with the highest citations. The Scientific Journal Ranking (SJR) website served as the reference for these journal rankings. Eight articles are classified in Q1, and seven in Q2, (Table 3), while five articles have not yet been assigned through the SJR website.

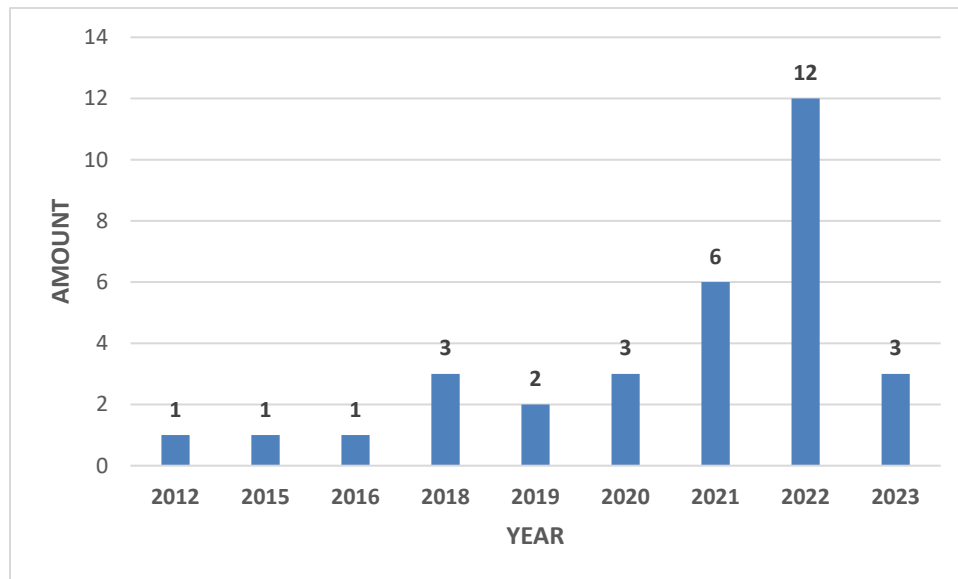


Figure 2. The selected OTEC-related articles by year published.

Table 3. The list of Q1 and Q2 journals with published OTEC-related articles.

Journal	Publica-tions	Percentage (%)	Scopus Quartiles
Journal of Cleaner Production	1	6.7	Q1
Journal of Marine Science and Technology	1	6.7	Q1
Energies	3	20	Q1
Renewable and Sustainable Energy Reviews	1	6.7	Q1
Sustainability (Switzerland)	2	13	Q1
Renewable Energy	1	6.7	Q2
Processes	1	6.7	Q2
Geosciences	1	6.7	Q2
Journal of Marine Science and Engineering	3	20	Q2
Journal of Ocean Engineering and Marine Energy	1	6.7	Q2

Methodology Utilised

All the selected publications were classified as quantitative studies using experimental research and survey methods in terms of research methodology. Most articles applied the advanced technology of Geographic Information System (GIS) to analyze and display geographically referenced information of the study area. Meanwhile, several papers extracted the primary data

from GIS product servers too. This procedure facilitated ease of the process and reduced time consumption. Other studies have undertaken economic analyses to assess and predict the viability of OTEC plants. These economic evaluations considered the calculations and forecasts of capital expenditure (CAPEX), operational expenditure (OPEX), and the levelized cost of electricity (LCOE).

Additionally, multi-criteria decision-making (MCDM) is another method that has been documented in this study. In the context of OTEC studies, MCDM helped researchers draw conclusions and identify suitable site selection for OTEC plants. Finally, sensitivity analysis has also been employed to forecast the uncertainties or possible effects based on the study findings. The distribution of techniques used is illustrated in Figure 3, indicating GIS analysis, economic analysis, MCDM, and sensitivity analysis.

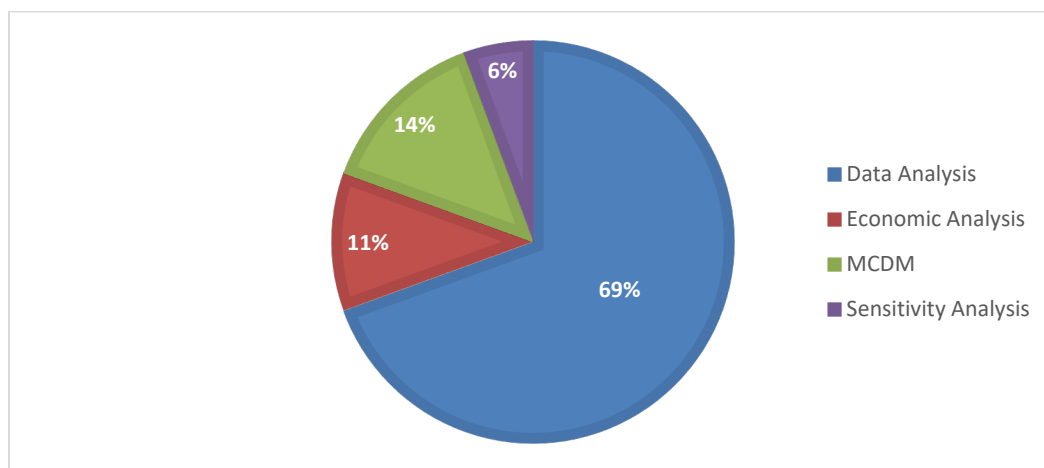


Figure 3. The techniques used in the included articles.

Research Topic

We found several criteria and/or parameters used in the published works on ways to determine site selection and suggestion on the type of OTEC plant in the selected OTEC sites. A temperature differential of at least 20 °C was considered as the fundamental requirement to build a commercially viable OTEC plant. The availability of temperature gradient is directly associated with the seawater depth at the chosen site. All the studies focused on temperature differences at depths ranging from 500 m up to 1,500 m, aiming to shorten the length of the cold seawater pipelines while achieving the minimum required temperature gradient. A shorter pipeline for an OTEC plant significantly reduces its capital expenses.

Of the selected publications, 11 articles addressed the potential of OTEC in Sabah waters. The bathymetry of Sabah's coast supports the development of an onshore, offshore, or fixed platform OTEC plant. The potential sites for OTEC plant are Layang-Layang Island (Fahmie et al., 2018; Thirugnana et al., 2021), Sipadan Island (Fahmie et al., 2018), Tawau (Thirugnana et al.,

2021; Waseda et al., 2021), Gumusut-Kakap (Fahmie et al., 2018), Banggi Island, (Fahmie et al., 2018; Thirugnana et al., (2021), Semporna (Waseda et al., 2021), Kunak (Waseda et al., 2021), Lahad Datu (Waseda et al., 2021) and Kalumpang (Thirugnana et al., 2021; 2023). These translate into an estimated total OTEC potential areas in Sabah of approximately 92,301 km² within the country's Exclusive Economic Zone (EEZ) which can produce up to 105,000 MW of renewable power (Thirugnana et al., 2021; Figure 4).

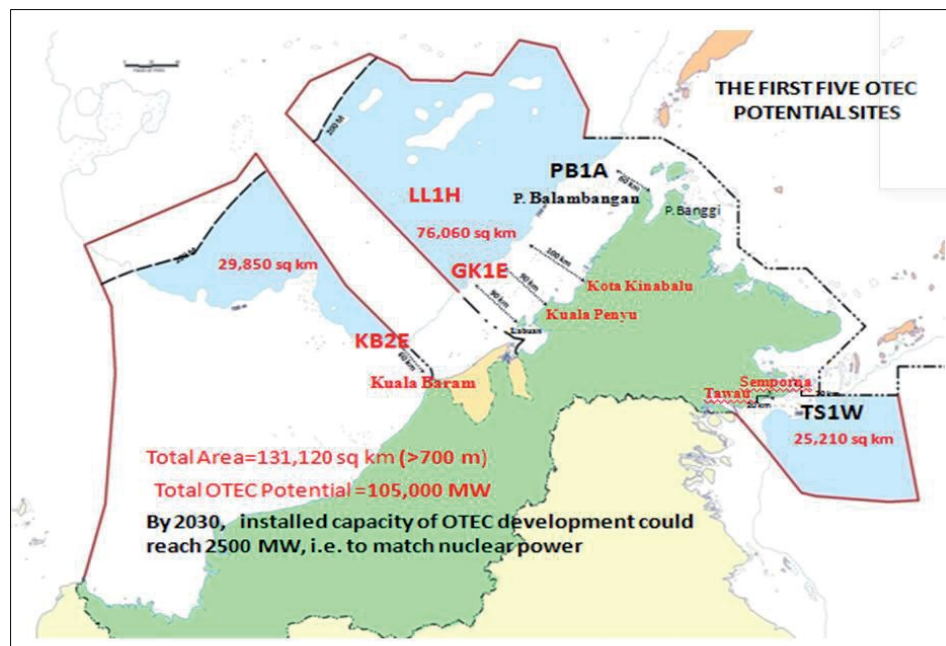


Figure 4. The OTEC resource potential in Malaysia, particularly in waters deeper than 700 m (after Jaafar, 2012). The blue highlighted areas represent sites with seawater depths greater than 1000 meters (<https://www.jodc.go.jp/>)

Discussion

Development of Potential OTEC Plant in Sabah, Malaysia

This study considered five potential sites and identified the type of OTEC plant installation for each location. These sites are strategically distributed across the northeast and southeast regions of Sabah, Malaysia, encompassing Layang-Layang Island, Mantanani Island, Mengalum Island, Kalumpang, and Tawau. The importance of site selection in OTEC projects influences the effectiveness and success of OTEC facilities. The site selection process is influenced by a multitude of circumstances, prompting researchers to explore various facets of it.

Fahmie et al. (2018) proposed the installation of a 4 MW fixed platform type at Layang-Layang Island. According to (Tasnim, 2023), Layang-Layang Island has the nearest deep seawater intake, which is 1.8 km from the closest coastline area, with a depth of 731 m. Thus, this plant is significant for renewable energy generation, despite its remote location – it is approximately 300

km northwest of Kota Kinabalu – and has no permanent human inhabitants. Layang-Layang Island was a thriving hub for socio-economic pursuits, such as deep-sea fishing, scuba diving, and tourism (Meng et al, 2024), prior to Covid-19 pandemic (DE, 2024b). Recent proposals, including Tasnim (2023), underscored this island to lead Malaysia's renewable energy transition. The proposal for a 1 MW onshore OTEC plant emphasized harnessing the island's plentiful deep-sea waters for electricity output while protecting the ecosystem. This initiative seeks to establish a sustainable power source to meet energy demand.

In contrast, nearby islands such as Mantanani Island have a partially indigenous population and mariculture industries such as abalone and seaweed cultivation. Hence, the island is poised for an energy transition of a 10 MW offshore fixed platform OTEC system. This solution improves energy capacity and blends in well with the dynamic socioeconomic and ecological environment of the island. These initiatives set a good approach being used throughout Sabah to capitalize on OTEC resources for clean and renewable energy production. The consideration of the OTEC offshore system due to the facility's strategic location, situated approximately 78 km from the nearest coast, and the water intake is 710 meters deep. Meanwhile, Thirugnana et al. (2023) conducted a performance analysis on a commercial OTEC plant situated at Kalumpang. Their study proposed a 10 MW capacity using the plate-type heat exchangers, the Rankine cycle, and anhydrous ammonia as the working fluid. Through the findings, future study could concentrate on maximizing hybrid-OTEC systems for 2.5 MW or 10 MW commercial plants, meeting carbon net-zero goals. Moreover, the extension of these results could generate an economically viable of OTEC Malaysian Model. According to Tasnim (2023), Kalumpang is strategically located 14.86 km from the deep seawater intake, and its water intake is 699 m below the surface, making the onshore system a viable option. Through an onshore OTEC system, it could synergize with the main socio-economic activity which is centered around palm oil plantations.

Another potential site for an onshore OTEC system is Mengalum Island; it has a deep seawater intake that is 691 m deep and approximately 44 kilometers between the nearest coast and the deep seawater intake. Hence, the development of an offshore OTEC system with a 10 MW capacity is possible. This island presents socio-economic activities, especially as a premier deep-sea fishing destination. This maintains a healthy balance between environmental conservation and economic growth, which could well become the lead in the field of sustainable energy technology through the onshore OTEC system.

Figure 5 presents a summary of the suggested OTEC types in the potential OTEC sites in Sabah, Malaysia.

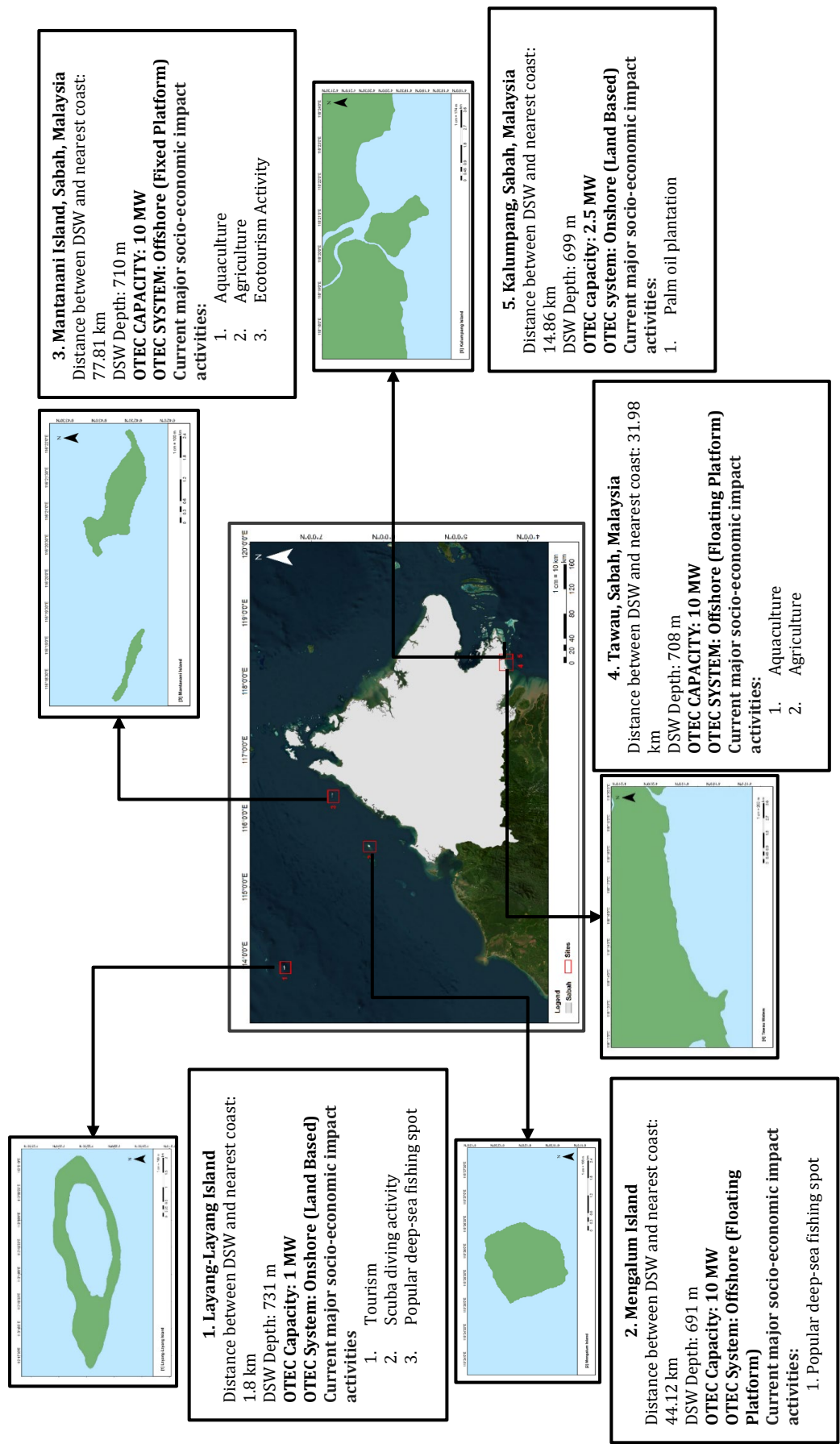


Figure 5. A summary of OTEC types in the five potential OTEC sites in Sabah, Malaysia.

The Current Progress of OTEC Development in Malaysia

A team of researchers are investigating the viability of establishing and commercializing the first OTEC plant in Malaysia, focusing on Sabah. The Science and Technology Research Partnership for Sustainable Development (SATREPS) is providing financial support for this project within a five-year timeframe, 2019-24 (SATREPS, 2024). Since the first OTEC Stakeholder Engagement was held in February 2023, continued efforts have been carried out to interact with various stakeholders regarding Malaysia's first OTEC facility, in addition to research and laboratory analyses (Tasnim et al., 2023). Following the engagement, a number of industry partners have pledged to support the first Malaysia-Japan OTEC Association's (MJOA) initiation. Additionally, other engagements including with the President of the Malaysian Bottled Water Association (MBWA) for data exchange related to the local and international bottled water sectors. This engagement seeks to increase public awareness of the ongoing efforts to commercialize the first OTEC ecosystem in Malaysia. Furthermore, a 2.5 kW Hybrid-OTEC Testing Plant has been established at the International Institute of Aquaculture and Aquatic Sciences (I-AQUAS), Universiti Putra Malaysia (UPM), located in Teluk Kemang, Port Dickson, Negeri Sembilan (Figure 6).



Figure 6. The hybrid-OTEC testing plant at (I-AQUAS), UPM Teluk Kemang, Port Dickson, Negeri Sembilan, Malaysia.

Conclusion

An OTEC plant requires a minimum of 20°C temperature difference between both surface and deep seawater intake points for optimal energy production. This temperature difference is the main criterion in OTEC site selection and type of plant. In this study, the Sabah Trough's surface and ground temperatures are consistent with the core principles of OTEC system. This study identified three potential OTEC plant types, namely (i) onshore (land-based), (ii) offshore (floating) and (iii) fixed platform. A total of five potential sites were determined throughout this study, with three sites located in the northeast, and two in the southeast of Sabah. The sites are Kalumpang, Tawau, Mengalum Island, Mantanani Island and Layang-Layang Island. Site location of OTEC has been considered based on the distance between the deep seawater resources and the nearest coast, depth of seawater as well as the major socio-economic impact activities within the area*. As for the type of OTEC plants, we propose a 1 MW onshore OTEC plant at Layang-Layang Island and a 2.5 MW OTEC plant for Kalumpang. For Tawau and Mengalum Island, we propose a 10 MW offshore floating platform to be constructed surrounding the coastal area at these sites. A 10 MW OTEC fixed platform plant is proposed for Mantanani Island.

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***Editors' Note:** As of December 2024, Layang-Layang Island remains closed to public and there are currently no socio-economic activities on the Island.