

Screening of peat water microalgae as natural feed candidates for sustainable local aquaculture

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Abstract. Sustainable aquaculture necessitates the development of feed using protein sources derived from natural materials. The challenge in aquaculture feed is the shortage of raw materials that can enhance the nutritional value of fish. Most research predominantly concentrated on utilizing artificial feed to improve fish growth and survival. Most research predominantly concentrated on utilizing artificial feed to improve fish growth and survival. Therefore, this study aimed to screen peat water microalgae as a potential natural feed source for local fish farming specific to Central Kalimantan. There are a few methodologies employed in this study that involve collecting samples from peat swamp water, isolating microalgae, and identifying their morphology, followed by cultivation. Bold's Basal Medium and BG-11 Medium were the media used for single-cell isolation and serial dilution. During the sample collection, several microalgae were identified in peat waters, including Micrasterias mahabuleshwarensis, Desmidium gravillei, Euastrum insigne, Pleurotaenium trabecula, Euastrum germanicum, Pleurotaenium nodulosum, Pleurotaenium nodosum, Closterium lunula, Micrasterias foliacea, Micrasterias anomala, Micrasterias americana, Anabaena sp., Xanthidium sp., Cosmarium reniforme, and Nitzchia sp. The isolates obtained from a single-cell isolation method comprised Chlorella, Pinnularia, Nitzchia, Scenedesmus, Chlorococcum, Cyanobacteria, Ankistrodesmus falcatus, Ankistrodesmus arcuatus, and Monoraphidium. The isolates acquired from the serial dilution method included Chlorella, Chlorococcum, and Cyanobacteria. Chlorella and Cyanobacteria were the microalgae that can be cultivated due to their ability to be purified in BG-11 medium. Thus, Chlorella emerges as the optimal candidate for aquaculture because of its rapid growth, axenic culture, and significant potential for aquaculture. Chlorella presents substantial possibilities for use in fish feed. It is safe for fish and does not negatively impact their growth, survival, or immune function. It stands out as a safe and sustainable choice for aquaculture, devoid of harmful chemicals and antibiotics. Therefore, this research promoted *Chlorella as* a feed candidate for sustainable aquaculture.

Key Words: peat water, fish, microalgae, feed, screening.

Introduction. Central Kalimantan has a large freshwater fisheries potential consisting of lakes, rivers, and swamps (Elvince & Aunurafik 2020). The total production of freshwater fish living in Central Kalimantan waters in 2020 was 44.515 tons consisting of 270 types of fish (Statistic Data and Information Center of the Ministry of Marine Affairs and Fisheries 2022), such as haruan (*Channa striata*) (Prisca Pricilla et al 2023), pepuyu (*Anabas testudineus*), tambakan (*Helostoma temminckii*) (Santoso & Wahyudewantoro 2018), and Kapar (*Belontia hasselti*) (Agustinus & Minggawati 2021). The aquaculture business is one way to maintain local fisheries resources and is the key to food security in Central Kalimantan.

Aquaculture ideally uses ecological and sustainable technologies. Some aquaculture practices are considered unsustainable, such as overfishing for farmed fish feed, fishmeal and fish oil contaminated with pollutants, and high feed prices. Artificial feeds are one of the issues. Artificial feed has several disadvantages, such as expensive production costs and declined water quality conditions. Fishmeal and fish oil are derived from wild fish, but because catches are restricted in several ways, more sustainable alternatives and solutions are being searched for (Zlaugotne 2022). Sustainable aquaculture requires the development of feed with protein sources from plant-based materials (Albrektsen et al 2022).

The use of plant-based alternatives (soybean, corn, wheat) to fish oil raises issues for both the environment and human health. The benefits of eating farmed fish for human health are lessened since they lack two types of omega-3 fatty acids, a type of dietary polyunsaturated fat (McKuin et al 2022). Thus, feed nutrition plays an important role in the development of aquaculture production, so the need to provide feed sources that have nutritional standards is continuously needed (Hu et al 2023).

Several previous studies conducted by Maryani et al (2021) have examined the provision of strange leaves in feed, which affects the blood profile and survival of tilapia. Feeding *Cromileptes altivelis* with an addition of 0.2% hard milk wood plant *Alstonia acuminata* can enhance the growth rate while achieving a high survival rate (Syahailatua et al 2017). Ratnasari et al (2020), who studied the addition of catfish silage to the feed, found no significant effect on the survival of catfish. Yulintine et al (2023) discovered that the optimal feeding for the survival and growth of snakehead fish larvae involves using natural live feed, such as silkworms and Daphnia *magna*. However, live feeds present challenges such as poor nutrient composition, the uncertainty of mass culture, and high levels of pathogenic microorganisms (Melaku et al 2024). Thus, natural feed derived from plant-based materials with a high nutritional content could serve as the best alternative to artificial feed.

Microalgae can be used as a substitute for artificial feed to increase and develop aquaculture productivity (Ahmad et al 2022). Microalgae produce omega-3 and omega-6 (long-chain) poly-unsaturated fatty acids that are recognized as being essential in human nutrition (Taelman et al 2013). Microalgae are rich in active chemical compounds, such as polyunsaturated fatty acids (PUFA), pigments, polysaccharides, and vitamins. Microalgae used for aquaculture are *Chlorella*, *Tetraselmis*, *Skeletonema*, *Chaeteros*, *Scenedesmus*, and *Nannochloropsis* (Ahmad et al 2022). *Nannochloropsis* sp. and *Isochysis* sp. can be used as a substitute for fish oil and fishmeal in rainbow trout feed (Sarker et al 2020). Annamalai et al (2021) mentioned that tilapia fed a diet containing *Chlorella*, *Nannochloropsis*, and *Schizochytrium* could increase their weight by 69%, 58%, and 46%.

De Araújo et al (2024) conducted a screening of microalgae that mentioned *Arthrospira maxima* biomass was more suitable for the development of fish feed. The results strongly support the use of *A. maxima* biomass as an alternative ingredient and protein source to replace fishmeal in fish feed. A fish feed with a value-added algal diet signifies the effective utilization of feed by the fish. Twelve selected algae having high pigment content and nutritional value can be used as fish feed to enhance the growth performance and nutritional value of *Catla catla* (Hajong & Ramanujam 2020).

Several microalgae have been found in the peat swamp waters of Central Kalimantan, such as *Closterium*, *Micrasterias*, *Chlorella*, *Botryococcus*, *Cosmarium*, *Euastrum*, *Pleurotaenium*, *Chroococcus*, *Chlorogonium*, and *Euglena* (Tsuraya et al 2023). However, these microalgae have only been observed and identified to a limited extent. The observed peatwater microalgae can be further investigated as a candidate for local fish feed in Central Kalimantan since fish are involved in feeding on microalgae in the ecological food chain. Therefore, studies screening microalgae from peat water environments are crucial to properly exploiting microalgae in aquaculture and increasing sustainability. The purpose of this study is to screen peat water microalgae through identification, isolation, and cultivation which is then connected with existing literature to determine its potential as a fish feed candidate, especially for local fish cultivation.

Materials and Method

Microalgae sample collection. Microalgae were collected with the filtration of 50 liters of water using plankton net 25 μ m size from peat swamp waters around Palangka Raya University (2°13'17.44" S and 113°53'04.57" E), at three locations, a puddle behind the FMIPA UPR Building in the ditch in front of the UPR Fisheries and the peat waters next to the PPIIG UPR building, (Figure 1). The sampling locations were characterized by stagnant water with a slightly cloudy color. These were based on those with diverse types of microalgae. This is based on a preliminary study that found that stagnant and turbid water conditions contained few microalgae compared to clear and flowing ones. Microalgae in peat water behind the FMIPA building (stagnant or lentic) were more abundant than those in another location that flowed and were more brownish (Tsuraya et al 2023).

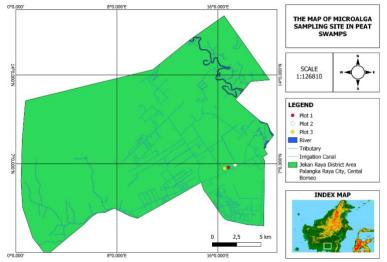


Figure 1. Map of microalgae sampling locations in peat swamp waters.

Microalgae isolation. The microalgae were pre-isolated by adding Walne medium, then incubated under constant illumination intensity at 60 mol $m^{-2}s^{-1}$ with 24-hour bright photoperiod using white fluorescent lamp lighting at a temperature of 25 ± 2^{0} C for 3 weeks (Fernandez-Valenzuela et al 2021). The microalgae that had grown were then isolated. There are two isolation methods used, namely single-cell isolation and multi-stage dilution method (Andersen 2005). The former was carried out by storing one cell from the sample in a drop of sterile culture medium, then taking another cell and transferring it to the second drop of sterile medium. This step was repeated until one algal cell could be placed in the last isolation container (Odeh et al 2023). The microalgae were moved to a 24-well plate and then waited until they grew approximately 2-3 weeks. The latter was done by diluting the sample to 10^{-3} or 10^{-4} (Kim 2015a, 2015b). The last dilution was added with Basal Bold Medium (BBM) or Blue Green (BG) 11 to grow the microalgae. As much as 1 mL of the sample was spread on BBM or BG 11 agar medium. The isolated sample was then incubated again. The growing sample was observed under a microscope to ensure that the microalgae were homogeneous and purified again on a new solid medium.

Morphological identification. Microalgae samples and isolates grown on solid media were identified morphologically to the genus level. Microalgae cells were observed based on cell size, shape, chloroplasts, pyrenoids, and flagella under a microscope with a magnification of 400x. Morphological identification followed Evans and Prescott (1956) and Bellinger and Sigee (2015).

Microalgae cultivation. Microalgae isolated in solid or liquid medium were cultivated first in 100 ml bottles as a starter. The microalgae starter culture was measured for cell density up to 10^6 cells ml⁻¹. The culture was transferred into BBM or BG-11 medium for cultivation. The cultivation used 250 ml glass bottles with the starter culture and medium ratio of 1:100 in a volume of 200 ml. Microalgae were then exposed to light for 24 hours which was

incubated at room temperature. Microalgae culture was aerated as well. The growth was observed every 24 hours based on optical density (OD) measurements using a 680 nm spectrophotometer calibrated using the medium sample without culture as a blank (Gour et al 2016).

Results and Discussion. The microalgae collected from 3 locations of the peat waters comprised four species of *Micrasterias, M. mahabuleshwarensis, M. foliacea, M. anomala,* and *M. Americana, two species of Euastrum, E. insigne and E. germanicum, three species of Pleurotaenium, P. trabecula, P. nodulosum, and P. nodosum,* and several other groups represented by one species, *Desmidium gravillei, Closterium lunula, Anabaena* sp, *Xanthidium* sp., *Cosmarium reniforme,* and *Nitzchia* sp (Figure 2). The most microalgae were found in location 2, which is a puddle behind the FMIPA UPR Building. It could result that the conditions of the peat swamp waters used as sample locations are quite similar and close together. Microalgae directly observed and identified were large one and clear to be observed at a magnification of 100x and photographed under a microscope. Data collection for observation and identification was also based on the morphology of microalgae. However, the small size and the debris covering the microalgal cells made the observation difficult to do, The morphological characteristics and potential in microalgae obtained from sample collection are presented in Figure 2.

Micrasterias have two semi-cells and are demarcated by a deep median groove. Cells are small to large, usually solitary, with a deep median constriction (isthmus) (Levanets & Janse van Vuuren 2023). It is important for model systems for cytomorphogenesis and cellular plasticity studies (Meindl 1993). It is used as an aquatic bioindicator as well. Micrasterias is found in the stomach of fish, such as *Tilapa zillii* and *Sarotherodon melanotheron* (Atindana et al 2016). Fujibayashi et al (2021) stated that in algae feeding on the bivalve *Corbicula* spp. Found in the stomach content of these bivalves, *Micrasterias hardyi* became the third most abundant algae found. However, this species does not contribute to the growth of bivalves because the abundance of this algae in the stomachs of these consumers may inhibit effective carbon transfer.

Micrasterias mahabuleshwarensis is a freshwater green-alga species first described in 1863 by J. Hobson. Cell longer than wide ($165 \times 142 \mu m$; isthmus: $26 \mu m$); deep median constriction, fully open median incision, triangular 5-lobed semi-cell; semi-rectangular incisions between the polar lobe and the lateral, open and deep; lateral lobes with pyramidal sinus, divergent lobes with entire apices; polar lobe with divergent processes with entire apices, apical margin of the polar lobe is straight; polar lobe of the same size as the sides; serrated cell margin. Habitat this alga prefers oligotrophic to mesotrophic lakes and bogs (Silva & Felisberto 2015).

Micrasterias folicea is a filamentous form that is quite different from all other species within the genus. It is the only species of *Micrasterias* whose cells are permanently attached to form a ribbon-like chain, which may comprise 2-100 cells. The cells are nearly square (sub-quadrate) and exhibit significant sinus constrictions. An apical (polar) lobe is present in each semi-cell, whereas the other two lobes are lateral. The lateral lobes are further divided into smaller lobes and lobules by incisions of different depths. The slender polar lobes have two pointed extensions at either end. The polar extensions interlock where neighboring cells are joined. The cells have an isthmus of 13–16 μ m and measure 70–80 μ m in length and 65–80 μ m in width. Some species have varying-sized spines, processes, or protuberances. Each semi-cell typically has one chloroplast with a few to many pyrenoids. The nucleus is localized in the isthmus. This species is found in lentic water bodies, such as ponds, marshes, wetlands, and different kinds of swamps, including peat swamps (Levanets & Janse van Vuuren 2023)

Micrasterias anomala is a unique and unusual plant that comes in several variants. It resembles *Micrasterias americana* and *Xanthidium armatum*. According to Cushman (1908), *Micrasterias americana* has a flat, variable-shaped cell body that is longer than wide and has a strong contriction at the center. Each semi-cell has several notches, and the cell wall has numerous small spines. *Micrasterias americana* can be cultivated in Waris medium (Ueda & Yoshioka 1976). *Micrasterias* cells were also grown in semi-sterile Erlenmeyer flasks using liquid Desmidiacean media (Schlösser 1982).



Figure 2. Results of Observations of Microalgae Samples from Peat Water. (Description left to right: Micrasterias mahabuleshwarensis, Desmidium gravillei, Euastrum insigne (Row 1) Pleurotaenium trabecula, Euastrum germanicum, Pleurotaenium nodulosum (Row 2) Pleurotaenium nodosum, Closterium lunula, Micrasterias foliacea (Row 3) Micrasterias anomala, Micrasterias americana, Anabaena sp (Row 4) Xanthidium sp., Cosmarium reniforme, Nitzschia sp (Row 5)) (scale: 10x). Desmidium grevillei has a dimension of 25.6-26.8 mm long and 34.5-48.8 mm wide. Isthmus 3.4-3.5 µm wide. Cells are octagonal in outline and elliptical in apical view. The sinus is widely open and shallow. Smooth cell wall with two mamillated thickenings on the semi-cell edges (Shakhmatov & Pavlovskiy 2019). D. grevillea is synonymous with D. cylindricum (Tomaszewicz et al 1993). The cells are united to form twisting filaments. A series of cells are alternatingly faced in a relatively broad frontal view and in a relatively narrow lateral view (and transitional phases in between) resulting in a filament with a seemingly varying diameter. Cells of this filamentous desmid genus have different shapes, an oval apical perspective and an octagonal shape. The sinus is shallow and extensively open (Shakhmatov & Pavlovskiy 2019). The cell has a smooth wall with two mamillated thickenings on the semi-cell borders. According to Tomaszewicz et al (1993), D. grevillea and *D. cylindricum* are interchangeable. Twisting filaments are created by joining the cells. A filament with a seemingly variable diameter is produced by alternating between a succession of cells in a relatively broad frontal view and a relatively narrow lateral view (as well as transitional phases in between). The morphology of the cells in this filamentous desmid species varies greatly. Some are oval and moniliform when seen in the end view, some are triangular, and some are guadrangular. Cells are wider than long, with or without a median incision but a slight median notch; walls at the poles of young semi-cells are unfolded or replicated (Evans & Prescott 1956). This microalga is collected in a central algal culture (CCAC) collection with a non-axenic culture. These microalgae are cultured in MiEB12 Ag (Andersen 2005).

Euastrum has an apex without spines or with a short, tooth-like spine at either side (Evans & Prescott, 1956). *Euastrum* cells are solitary, usually longer than broad, with deep median constriction (isthmus) where semi-cell walls overlap. The apical view is oval to elliptical (biradiate), often with the broadened middle region (rare triangular forms known). Each semi-cell usually has distinct apical and lateral lobes, with an apical lobe with an emarginate apex or apical incision. Cell wall smooth with scattered pores or variously ornamented with granules, verrucae, or short spinules. Usually, one chloroplast per semicell with one or more pyrenoids. Nucleus in isthmus (Anissimova 2021). Euastrum cells are solitary, typically longer than broad, and have semi-cell walls that overlap at the deep median constriction (isthmus). Often having a broader central area, the apical view is oval to elliptical (biradiate) (few triangular variants reported). Every semi-cell typically has separate lateral and apical lobes, with the apical lobe having an emarginate apex or an apical incision. Cell walls might be smooth with sporadic pores or decorated with small spinules, verrucae, or granules. Typically, each semi-cell has one or more pyrenoids and one chloroplast. The isthmus's nucleus (Anissimova 2021). Euastrum insigne is a rare species and only sporadically found in oligotrophic, acidic bog pools (Bellinger & Sigee 2015). Euastrum germanicum has too little information about its morphology and characteristics, so it is difficult to identify and research. *Euastrum* can be cultivated in an MWC medium. The eel fish (Notopterus notopterus), which inhabit Sei Gesek Reservoir, are known to consume Euastrum. Euastrum is the sort of food that is only present in eel fish stomachs and is the food that is naturally picked for ingestion; it has a food choice index of 1 for eel fish (Kurniawan et al 2021). The intestinal contents of tilapia fish (Oreochromis niloticus, Sarotherodon galilaeus, and Coptodon zillii) were also discovered to contain Euastrum (Shalloof et al 2020).

Pleurotaenium has a middle constriction, and chloroplasts are grouped in longitudinal bands and have a large number of pyrenoids. Cells are elongated straight cylinders that occasionally slightly taper at ends (Bellinger & Sigee 2015). This species is synonymous with *Docidium ehrenbergii*, *D. trabecula*, and *Closterium trabecula*. Cells are 540 μ m long, 30 μ m broad, isthmus 30 μ m, and apex 20 μ m. *Pleurotaenium* is distinguished by elongated cylindric cells (11-15 longer than wide), which are round when viewed from above and typically have a shallow sinus due to a minor constriction in the middle. There is room for a large, globular vacuole at the cell apex because the chloroplast is shaped like longitudinal, parietal bands. According to Shukla et al (2008), semi-cylindric cells have a single, distinct basal inflation and, in rare instances, a single undulation above it. They also have straight or slightly convex lateral edges, truncately rounded, tubercle-free apices, and a colorless, punctate cell wall. These microalgae were collected in a central

collection of algal culture (CCAC) with non-axenic culture. *Pleurotaenium trabecula* is found in the stomach of debit fish (*Rasbora tawarensis*) (Muchlisin et al 2015). *Pleurotaenium* is also found in the gut and stomach of mackerel (*Rastrelliger kanagurta*) (Hasibuan et al 2025).

Closterium lunula has a large cell body of 1000 µm long and 120 µm wide, innerside nearly straight, outer side convex, tapered at both termini (broad circle, slightly inversed), cell wall smooth, transparent, without bands, chloroplasts with many pyrenoids. *Closterium* has 2 axial chloroplast-bearing longitudinal ridges, chloroplasts in either horn of the cell, and pyrenoids conspicuous, usually in an axial row (Evans & Prescott 1956). It has a crescent shape with two chloroplasts containing pyrenoids. Cells are slightly or strongly curbed with tapering ends (Bellinger & Sigee 2015). According to Hur et al (2015), *Closterium* can be cultivated in Modified Bold 3N Medium (MB3N). Based on KMMCC, *Closterium* medium is Jaworski's medium. Studies on the cultivation of *Closterium* as a natural food have never been conducted, but *Closterium* is also reported as a food type found in the stomach of Bilih fish (*Mystacoleucus padangensis*) (Suryanti et al 2017).

Anabaena sp. is a tiny, bead-like individual. This group can combine to create lengthy chains that resemble beaded necklaces. The cells are frequently observably green. Heterocysts are specialized cells that allow this genus to fix nitrogen. Heterocytost cells might not be apparent in an Anabaena chain (Prasanna et al 2006). Anabaena can be cultured in BG-11 medium (Waditee-Sirisattha et al 2012). Anabaena was inoculated in Watanabe's liquid medium in culture flasks was incubated at 28-30°C for 10 days under continuous light, produced by fluorescent white bulbs of 120 cm length and fluorescence illumination (5500-6500 lux) (Fadl et al 2020). However, dietary Anabaena up to 15% did not impair nutrient utilization and supported normal growth performance in C. catla fingerlings (Mule et al 2024).

Xanthidium has a semi-cell apex that has noticeable spines; if there is a face protuberance, it has a single, big, low swelling, and the wall is thicker and frequently pitted or punctured (Evans & Prescott 1956) *Xanthidium basidentatum* has an apex of semi-cell furnished with conspicuous spines, facial protuberance (if any) one large low swelling, the wall thickened here and often fitted or punctate. Every semi-cell angle is plagued by a bundle of spines. There are granules near each of the basal angles. Xanthidiums have been isolated in Jaworski's medium (Hur et al 2015).

Cosmarium elegantissium are unicellular algae that have a variety of shapes, including flattened semicells that are hemispherical, spherical, ellipsoidal, rectangular, pyramidal, or kidney-shaped, and a constriction of the center of the cell body that is typically longer than wide. They also lack an apical identification. Cell margin that is occasionally granular but lacks spines (Evans & Prescott 1956). The median groove is very narrow, over all cell shape is ovoid to rounded, sometimes with slightly flattened sides or ends. No spines or extended processes are present but the cell wall may have markings (Bellinger & Sigee 2015). *Cosmarium* can be cultured in Jaworsky's medium (Hur et al 2015) and modified in Bold's Basal Medium (Daneshvar et al 2007). obtained from Merck, Germany. *Cosmarium* obtained from the batch experiments revealed the ability of algal species to remove the dye.

Nitzschia has two chloroplasts per cell, one on either side of the central axis. Two plate-like chloroplasts, one above and one below the central. The vast genus *Nitzschia* contains cells that might be spindle-shaped, elliptical, narrow linear, or sigmoid when viewed from the valve. Some species have slightly constricted the valve center. Each valve's raphe is shifted to one margin and is diagonally opposite the other valve. One edge of each valve displays the raphe, and each valve's raphe is diagonally opposite the other. The actual raphe structure is a canal held up by bars that resemble carinal dots. Cells are usually solitary but can form stellate colonies as well (Bellinger & Sigee 2015).

Nitzschia identified in this study have slightly different characteristics. The chloroplasts are dark green with clear white cytoplasm. *Nitzschia* usually has brown chloroplasts. This cell also does not have visible stripes like *Nitzschia* in general. Numerous *Nitzschia* species are known to be markers of water contamination or organic enrichment. Although they often exist alone, this group can also live in mucilage tubes or form stellate colonies. Two plastids are often seen in cells, one at each pole. *Nitzschia* has a fibular raphe

system and is typically located on or close to the valve surface's edge. The raphe is on the opposite margins of the two valves of a frustule (nitzschioid symmetry) (Lowe 2003).

Microalgae isolation was carried out using two methods, namely single-cell isolation and multilevel dilution. Microalgae were isolated as single cells, and their growth was observed daily. Microalgae growth can be seen by the naked eye with a green-yellow color on day 14 (Figure 3). The observations used 100x magnification on a microscope. However, *Micrasterias, Closterium, Xanthidium,* and others could not grow and died. These microalgae cannot be grown in growth media for microalgae isolation, such as BG-11 and Bold's Basal Medium. A list of microalgae identified from the field sample and the ability to grow when the isolation is conducted is shown in Table 1.

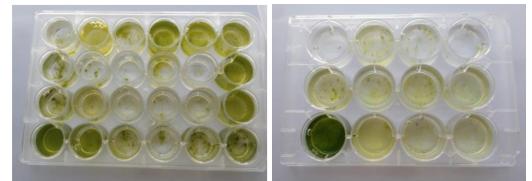


Figure 3. Microalgae obtained from single-cell isolation grew in well-plate (left: 24 well with BBM medium; right: 12 well with BG-11 medium)

Table 1

Microalgae	<i>Growth Ability in BBM and BG- 11 Medium</i>	Growth medium	Potential for aquaculture
Micrasterias	-		_
mahabuleshwarensis		Desmidicacean (Schlösser	
M. foliacea	-	1982)	-
M. anomala	-		-
M. americana	-		-
Euastrum insigne	-	MWC Medium	-
E. germanicum	-		-
Pleurotaenium trabecula	-	-	-
P. nodulosum	-	-	-
P. nodosum	-	-	-
Desmidium gravillei	-	MiEB12 Ag (Andersen 2005).	-
Closterium lunula	-	Jaworski's, MB3N (Hur et al 2015)	-
<i>Anabaena</i> sp	\checkmark	BG-11. Watanabe's Medium (Fadl et al 2020)	\checkmark
<i>Xanthidium</i> sp	-	Jaworski's (Hur et al 2015)	-
Cosmarium reniforme	-	Jaworski's (Hur et al 2015)	-
<i>Nitzchia</i> sp	\checkmark	Diatom Medium	-

List of microalgae from field sample

(-) Not grow (\checkmark) Grow ; (-) Not potencial (\checkmark) Potencial.

The microalgae that grew were those accidentally taken using a pipette and then grown in a well plate. The microalgae obtained from single-cell isolation were *Chlorella*, *Pinnularia*, *Nitzschia*, *Scenedesmus*, *Chlorococcum*, *Cyanobacteria/Oscilatoria*, *Anabaena*, *Spondylosium*, *Ankistrodesmus falcatus*, *A. arcuatus*, and *Monoraphidium* (Figure 4). Nevertheless, these microalgae are not pure and are still mixed in the well (nonaxenic). Purification is done by observing under a microscope, then rinsing the glass object with water and placing it on the conical bottom tube. Moreover, microalgae isolated by dilution method can also be observed on day 14 (Figure 5). *Chlorella* and *Chlorococcum* were successfully isolated by multilevel dilution (Figure 6). *Chlorella* grow in an axenic culture without contaminants. A list of Microalgae from sample isolation is shown in Table 2.

Table 2

Microalgae	Growth Ability in BBM and BG-11 Medium	Axenic Culture	Potencial for Aquaculture
Chlorella	\checkmark	\checkmark	\checkmark
Pinnularia	\checkmark	-	-
Nitzschia	\checkmark	-	-
Scenedesmus	\checkmark	-	\checkmark
Chlorococcum	\checkmark	-	\checkmark
Cyanobacteria	\checkmark	\checkmark	\checkmark
Anabaena	\checkmark	-	\checkmark
Spondylosium	\checkmark	-	-
Ankistrodesmus falcatus	\checkmark	-	\checkmark
Ankistrodesmus arcuatus	\checkmark	-	\checkmark
Monoraphidium	\checkmark	-	-

List of microalgae from sample isolation

(-) Not grow (\checkmark) Grow ; (-) Not axenic (\checkmark) axenic.

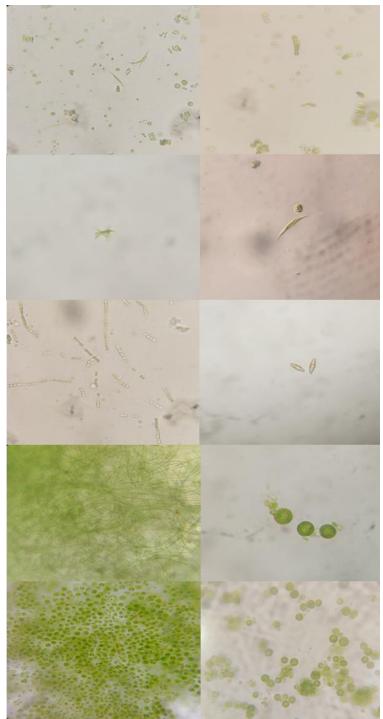


Figure 4. Microalgae Isolation from Peat Waters Isolated in BBM and BG-11 Medium (Line 1: Mix culture; line 2: Ankistrodesmus falcatus, Ankistrodesmus arcuatus; line 3: Anabaena, Pinnularia; line 4: Filamentous Cyanobacteria, Chlorococcum, line 5: Chlorella) (scale : 40x).

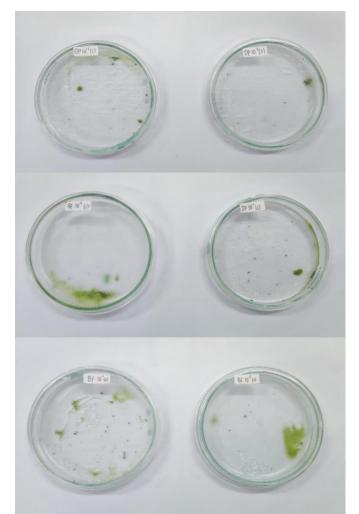


Figure 5. Microalgae obtained from the dilution method grew in the Petri dish after 14 days of incubation.

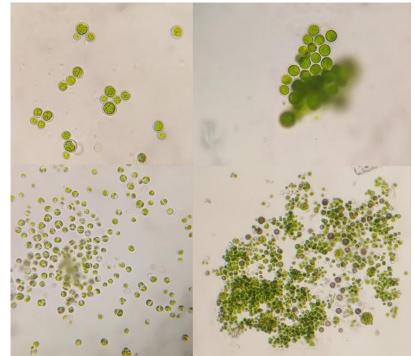


Figure 6. Microalgae isolated by dilution method, *Chlorella* (line 1) and *Chlorococcum* (line 2) (scale: 10x).

The two microalgae (*Chlorella* and Cyanobacteria) were cultured in a conical bottom tube for approximately 14 days, and the results are presented in Figure 7. *Chlorella* can grow quickly, as indicated by a green color. The large conical bottom (50 ml) becomes a better cultivation site for microalgae than the small one (15 ml). The large conical bottom tube has a larger surface and air volume, making the microalgae easier to use oxygen and air circulation. Microalgae need a large area and sufficient oxygen for growth. Transferring the microalgae to a conical bottom tube is to acclimatize and purify them from other contaminants.

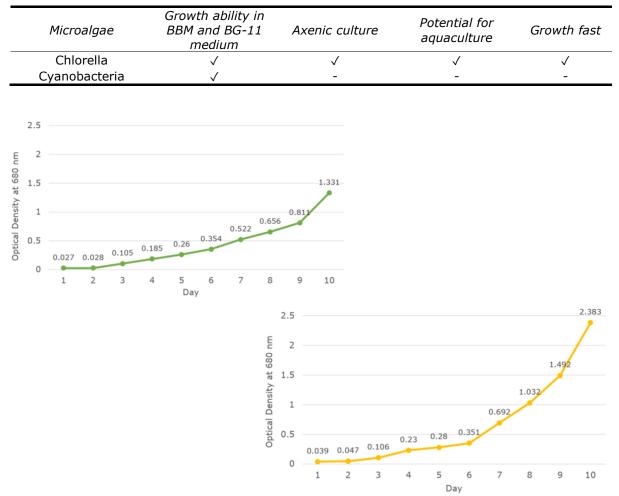


Figure 7. Microalgae were purified and moved to a conical bottom tube (left: *Chlorella*; right: Filamentous Cyanobacteria).

Both microalgae were then cultivated in BG-11 medium. This finding showed that only *Chlorella* sp grew easily in the BG-11 medium (Figure 8). Filamentous cyanobacteria can grow but are eventually contaminated with *Chlorella*. Chlorella was then harvested and put into a conical bottom tube for preservation. The microalgae were also re-cultured to observe their growth, which showed optical density (OD) results reaching 1.331 and 2.383 on day 10 (Figure 9). This number is quite normal in the BG-11 medium. Wong (2017) stated that *Chlorella* cultured in BG-11 reached 1.602 on day 14. A list of microalgae cultures after cultivation is shown in Table 3.



Figure 8. Microalgae *Chlorella* (left) and *Cyanobacteria* (right) from peat waters Cultivated in BG-11 Medium.



List of microalgae culture after cultivation method

Figure 9. Optical density growth of *Chlorella* for 10 days with 2 replications (Above: *Chlorella* replication 1; Below: *Chlorella* replication 2).

Chlorella is among the most common microalgae used in aguaculture as a direct feed or inserted into foods as an additive for different organisms (Sukri et al 2016). The biomass of Chlorella contains three main components: protein with a percent dry weight of 10 to 20%, carbohydrates, 40 to 70%, and lipids, 4 to 60%. DHA and EPA concentrations in Chlorella vulgaris are 36.53 and 123.46 mg g⁻¹ dry weight (Pratiwi & Pratiwy 2020) Studies also show that Chlorella is safe for fish and does not have adverse effect on growth, survival, and immune system function. Chlorella is also a safe and sustainable option for aquaculture, free from harmful chemicals and antibiotics (Aly et al 2024). Most species of Cyanobacteria cause fish mortality by producing toxins, so Cyanobacteria is not recommended to use aquaculture. Cyanobacteria such as Microcystis spp., Cylindrospermopsis raciborskii, Planktothrix (syn. Oscillatoria) rubescens, Synechococcuss, Anabaena spp., some Oscillatoria spp. etc. are toxic microalgae. Anabaena, Aphanizomenon, Oscillatoria, and Microcystis are toxic microalgae. According to histopathological analyses of fish died during cyanobacterial blooms, damage to the gills, digestive tract, and liver was the primary cause of mortality. The elevated pH produced by cyanobacterial photosynthetic activity before bloom collapse was most likely the source of the gill damage (Aklakur et al 2023)

Anabaena is also a good candidate for fish feed, but it is mentioned to be toxic. Before a challenge, Anabaena addition to the fish feed improves growth performance, protein content, serum biochemical parameters, and immune status of Nile tilapia (Fadl et al 2020). Moreover, Chlorococcum addition at a concentration of 3×10^6 cells mL⁻¹ is

suggested as the best food for the successful mass culture of the rotifer Brachionus calyciflorus. A nutrient-dense species with a greater crude protein (30.5%) and lipid (18.2%) content, *Chlorococcum* sp. can be utilized to produce the rotifer B. calyciflorus on a large industrial scale (Khan et al 2024). The Chlorococcum and Chlorella genera showed very low lipid storage (15.6-18.4%) and significant protein storage (46.4-48.8%). Ankistrodesmus contains 43.2-46.4% protein and 27.4- 32.2% fat. Scenedesmus was observed storing up to 48.8- 52.8% protein and 27.428.6% lipid (Abdinazarov et al 2023). Ankistrodesmus is another microalga successfully isolated but is difficult to purify and culture. This group can grow in BBM and COMBO medium (Okomoda et al 2021). There is a study that explains the potential of Ankistrodesmus as a candidate for fish feed. Ankistrodesmus gracilis has been studied as fish feed in Xinophorus maculatus, showing that the microalgae could be used as a functional ingredient because its biomass can improve nutritional quality and maintain high growth performance of the fish (Sipaúba-Tavares et al 2019). In addition, Sharifah et al (2016) stated that Ankistrodesmus sp. can be an essential tool for inhibiting the growth of *Streptococcus agalactiae* infecting fish. The present study has provided beneficial information on aquaculture development, particularly the use of nature-based food materials as fish food candidates. Chlorella is a microalga that can be cultured easily, has fast growth, and has the potential to be used as a local fish feed candidate. The type of *Chlorella* with the greatest potential for usage as a substitute source of fish feed is *Chlorella vulgaris*. In addition to being non-toxic and easy to grow, Chlorella vulgaris has a high nutritional content and an easily digestible cell wall. *Chlorella vulgaris* is suggested as a high-quality substitute protein source and an affordable fish feed (Albagami 2025)

Of all the microalgae observed from the sample collection, only a few microalgae can grow in the freshwater medium and have potential for aquaculture. It is necessary to study the specific medium required by these microalgae and try various growth media to isolate and cultivate each type of microalgae that has been obtained. The isolation and purification techniques of microalgae also need to be studied to get maximum results. *Chlorella* collected can only be identified by genus and needs molecular analysis to determine the species. *Chlorella* can be tried on local fish in Central Kalimantan with various treatments to determine growth, productivity, and fish immunity to disease. Among all the microalgae, *Chlorella* is the best candidate for local aquaculture because of its fast growth, axenic culture, and potential for aquaculture.

Conclusions. Among all the microalgae that have been selected from isolation, purification, to cultivation, *Chlorella* is the best candidate for local aquaculture because of its fast in growth, axenic culture, and potential for aquaculture. The results obtained from this study do not provide clarity on a more in-depth screening of microalgae as fish feed candidates. There is a lack of laboratory facilities, such as adequate microscopes and equipment for biochemical tests. This study suggests further analyzing the biochemical profile of *Chlorella* that has been successfully isolated and purified. Furthermore, it could be necessary to perform in vivo testing on certain native fish in Central Kalimantan, such as evaluating fish feed's digestibility and nutrition's impact on fish growth.

Conflict of interest. The authors declare that there is no conflict of interest.

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