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IDENTIFICATION AND EVALUATION OF LIPID-PRODUCING MICROALGAE FROM MARINE AND FRESHWATER ECOSYSTEMS FOR BIOFUEL PRODUCTION

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ABSTRACT

Microalgae represent a sustainable bioresource for renewable energy production, offering potential to reduce dependence on fossil fuels and mitigate climate change. Their lipid content, which varies by species and environmental conditions plays a critical role in determining biofuel yield and quality. Therefore, precise species identification and consideration of geographic origin are essential for optimal strain selection. The main objective of this study is to identify and characterise isolated microalgae based on their growth rate and lipid yield then select the most potential species, each marine and freshwater, for further study and applications. In this study, a total of nine microalgal isolates from marine and freshwater sources in Sabah were isolated and established in cultures. Marine microalgae were cultivated in Walne's Media (WM) while those of freshwater in Bold's Basal Medium (BBM). Molecular identification based on 18S ribosomal RNA sequencing identified eight different species which were revealed to be *Caecitellus parvulus*, *Caecitellus pseudoparvulus*, *Halamphora subtropica*, *Tetraselmis striata*, *Mychonastes afer*, *Nitzschia hantanense*, *Monactinus sturmii*, and *Pseudomuriella* sp. Growth characteristics were assessed via direct cell counting using a haemocytometer, biomass, and lipid productivities, as well as lipid content, were quantified gravimetrically. The specific growth rates of marine isolates were determined to be between 0.20 and 0.26 day^{-1} while those of freshwater were determined to be between 0.12 and 0.37 day^{-1}. During the exponential phase, marine isolates exhibited biomass productivities ranging between 1.55 and 6.57 mg/L/day while freshwater isolates ranged between 0.85 and 4.17 mg/L/day. The highest lipid yields were 51.9% for marine and 63.25% for freshwater isolates. These findings demonstrate that environmental origin influences microalgal growth and lipid accumulation under controlled culture conditions, supporting their potential application in biofuel production.

Introduction

Fossil fuels and natural gas remain the primary sources of global energy despite being non-renewable, leading to concerns over long-term energy security. Moreover, when combusted, they also generate large amounts of carbon dioxide, contributing significantly to global climate change. In response, policy makers and shareholders have initiated global efforts to reduce greenhouse gas emissions and transition toward alternative, renewable energy sources. As a result, extensive research has been dedicated in exploring sustainable, carbon-neutral energy options, of which, biomass has shown considerable promise.

Biomass can be derived from plants and microorganisms found in organic or waste matter (Khan *et al*., 2018) including microalgae, which are capable of being converted into liquid biofuels (Rony *et al*., 2023). Moreover, microalgae have gained recognition for its potential as a biofuel source due to its rapid growth rate, high lipid content, and ability to thrive in diverse environments (Van Lal Chhandama *et al*., 2023). Unlike traditional biofuel crops, microalgae do not compete with food production and can be cultivated on non-arable land using wastewater or salt water, making them a sustainable and efficient option for renewable energy production (Abdelfattah *et al*., 2023).

Lipids extracted from microalgal biomass can be converted into biofuels via a transesterification process. However, the quality and yield of biofuels derived from microalgae are heavily influenced by the species-specific lipid content and fatty acid composition (Khoo *et al*., 2023). Oleaginous microalgae such as *Chlorella* sp., *Botryococcus braunii*, and *Scenedesmus obliquus* have been reported to produce lipid yields ranging between 30% and 75% of their biomass, which exceeds the lipid content of conventional oil crops such as palm oil seed (35%) (Sajjadi *et al*., 2018). Therefore, the identification and cultivation of microalgal strains with high lipid productivity holds significant promise for biofuel applications yet remains underexplored.

Environmental conditions also strongly influence microalgal physiology, growth, and biochemical profiles (Kholssi *et al*., 2023). Factors such as light intensity, pH, temperature, nutrient availability, salinity, and carbon levels contribute to the diversity and adaptability of microalgal species (Khoo *et al*., 2023). As a result, geographic origin becomes a critical factor in species selection, as different habitats give rise to species with distinct growth characteristics and lipid accumulation profiles (Anggraini *et al*., 2018). Marine and freshwater ecosystems differ primarily in terms of salinity, with marine water typically at between 33 and 38 parts per thousand (ppt) while freshwater usually has a salt content of less than 1 ppt (Eilers *et al*., 1990). Different species exhibit varying degrees of tolerance to salinity changes. Marine and brackish microalgae have evolved various physiological and biochemical adaptations to tolerate the high salinity of seawater that would otherwise inhibit photosynthetic ability and growth. Besides salinity, the degree of turbulence, tidal changes, nutrient inputs, and fluctuating temperatures of these habitats may give rise to unique adaptations as well.

Studying microalgae found in Sabah is crucial as the region's unique tropical marine and freshwater ecosystems may foster distinct microalgal adaptations that could enhance lipid productivity. By focusing on locally sourced strains, researchers can optimise cultivation and biofuel production strategies tailored to the specific environmental conditions of Sabah, ensuring sustainability and economic viability while leveraging the region's rich biodiversity. Previous studies have focused on isolation and cultivation of marine species because the seawater used as a culture medium is more abundant, accounting for up to 97% of Earth's surface area.

On the other hand, freshwater species have a smaller water footprint due to their lower saline tolerance (Zhai *et al*., 2020; Mahata *et al*., 2022) despite some freshwater species having higher growth rates and being better accumulators of lipids such as *Neochloris oleoabundans* and *Scenedesmus obliquus* (Moreira *et al*., 2021). Even so, both sources have potential as lipid producers and are the subject of much intense research in recent studies (Penloglou *et al*., 2025; Kaur *et al*., 2025) and may lead to the discovery of a commercially viable species. This emphasises the importance of bioprospecting both local marine and freshwater environments to identify and cultivate microalgae with high lipid productivity for cost-effective and sustainable biofuel production.

This study aims to (1) isolate and identify microalgae from both marine and freshwater ecosystems in Sabah with potential for biofuel production and to (2) evaluate and compare their growth characteristics, biomass productivity, and lipid yield of microalgae under controlled culture conditions. The findings of this study will support the selection of high-performing local species suitable for large-scale cultivation, contributing to the development of microalgae-based biofuels and expanding the library of commercially viable microalgae.

Materials and Methods

Sample Collection

Water samples were collected from various local freshwater and marine ecosystems in Kota Kinabalu, Sabah. During sampling, a plankton net of a 20 µm mesh size was used to collect microalgae, which were then transferred into 250 mL plastic bottles for further analysis. At each sampling site, in-situ water quality with physical parameters including pH and salinity were measured using a portable NOYAFA NF-C600 water quality tester (Noyafa, Hong Kong). All collected samples were transported to the laboratory on ice to maintain sample integrity.

Marine water samples were enriched with Walne's Media while freshwater samples were enriched with Bold's Basal Medium (BBM) to provide favourable conditions for microalgal growth.

Isolation of Microalgae and Culture Establishment

The microalgae were isolated from the enriched water samples using the micropipette washing technique. Capillary pipettes were prepared by heating sterile glass Pasteur pipettes over a flame, following the protocols of Tsuchikane *et al*. (2018). The isolated microalgae were established as unialgal cultures and incubated in a growth chamber (Panasonic, Japan) maintained at 23°C, under a 16:8 hours light/dark photoperiod, with a light intensity of approximately 65 µmol photons m^{-2} s^{-1}.

Morphological Observation

The isolated stains were observed under a standard light microscope (OLYMPUS, USA) to assess their morphological features. Morphological identification was based on characteristics such as cell size, shape, pigmentation, cell arrangement, colony formation, motility, presence of flagella, whether filaments were branched or unbranched, as well as the presence of spines (Fawley & Fawley, 2020).

Molecular Identification

The isolated microalgae were identified based on amplification and sequencing of the 18S ribosomal RNA (rRNA) gene using the primer pair, 18SCOMF1 (5′-GCTTGTCTC AAAGATTAAGCCATGC-3′) and 18SCOMR1 (5′- CACCTACGGAAACCTTGTTACGAC-3′) (Zhang *et al*., 2005). Total genomic DNA was extracted from approximately 100 mL of microalgae cultures using Nucleospin® 96 Plant II kit (Macherey-Nagel, Germany), with slight modifications to the manufacturer's protocol (Andrew *et al*., 2022). PCR amplification was performed in a 20 µL reaction mixture containing 1 × GoTaq® Flexi Buffer (Promega, USA), 3 mM $MgCl_2$ (Promega, USA), 200 µM dNTP mix (Promega, USA), 1.25 U of GoTaq® DNA polymerase, 1 µM of each primer, and 50 ng of DNA template.

The reactions were carried out using a C1000 thermal cycler (Bio-Rad Laboratories, USA) with the following conditions: Initial denaturation step at 95°C for 2 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 5 minutes, with a final extension step at 72°C for 5 minutes. PCR products were analysed by electrophoresis on a 1.5% (w/v) TBE agarose gel stained with 4 µg/mL ethidium bromide and visualised using a GeneRuler 1kb DNA ladder (Promega, USA) as a molecular size marker.

Successfully amplified products were purified and sent for Sanger sequencing at Apical Scientific Sdn. Bhd. (Malaysia). After

sequencing, the raw DNA sequences were edited and trimmed using BioEdit Sequence Alignment Editor (v7.7.1) and MEGA (v11.0.3). The edited sequences were then analysed using the BLAST algorithm to identify similarities with existing sequences in the NCBI database.

Determination of Growth Characteristics and Growth Rate

The estimation of growth characteristics for the isolates was based on the number of cells in the culture per volume (mL) to measure cell density per volume (cells/mL). Cell density for all the microalgal isolates was monitored every three days for 30 days using direct cell counting with a hemacytometer under a light microscope (Tulashie & Salifu, 2017). To immobilise motile algal cells, one to two drops of 10% formalin were added to each sample. Cell density was calculated based on Equation (1) and used to construct growth curves for each isolate. The specific growth rate (μ) of each isolate was then determined using Equation (2).

$$\text{Cell Density (cells/mL)} = \text{Average count per square} \times \text{dilution factor} \times 10^4 \quad (1)$$

$$\mu(t) = \frac{ln\, x - ln\, x_0}{t - t_0} \quad (2)$$

where x_0 and x represent the number of cells per volume (mL) at time t_0 (start of cultivation) and t (end of cultivation), respectively.

Determination of Biomass Productivity

Biomass productivity of microalgal cultures was determined during both mid-exponential and stationary growth phases (Rahman *et al*., 2020). Biomass was harvested from approximately 48 mL of culture on day 7 and 9 for mid exponential phase and day 17 and 22 for the stationary phase.

The cultures were centrifuged at 4,000 ×g for 20 minutes to obtain the wet algal biomass, which was rinsed twice with distilled water. The biomass was then frozen overnight at -80 ℃ and subsequently freeze-dried for three days using a Freeze Dry System (LABCONCO, USA) to obtain the dry biomass weight. Biomass productivity (g/L/day) was calculated gravimetrically using Equation (3) (Nascimento *et al*., 2013).

$$\text{Biomass productivity (g/L/day)} = (X_2 - X_1) / (t_2 - t_1) \quad (3)$$

where X_1 and X_2 represent the biomass dry weight concentrations on day t_1 (start of cultivation) and t_2 (end of cultivation), respectively.

Determination of Lipid Yield and Lipid Productivity

Similar to biomass productivity, lipid yield and lipid productivity of microalgal cultures were determined during both the mid-exponential (day 9) and stationary (day 17 to 22) growth phases. Lipid extraction was carried out using a chloroform:methanol:water (1:1:1) solvent system based on the Bligh and Dyer method (Bligh & Dyer, 1959). About 100 mg dried microalgal biomass was transferred into a falcon tube and chloroform, methanol, and water were added in a 1:1:1 ratio.

The mixture was vortexed thoroughly and left to stand at room temperature for two hours until two distinct layers formed. The bottom organic layer was transformed into a clean pre-weighed microcentrifuge tube, then, the upper aqueous layer (methanol and water) was carefully discarded. Residual methanol in the lower organic layer was removed using a vacuum concentrator (V-AQ mode) (Eppendorf, Germany) at 45°C for two hours.

The crude lipid extract was then weighed and lipid yield was calculated using Equation (4). Lipid productivity (g/L/day) was calculated by multiplying the lipid yield with biomass productivity, as shown in Equation (5).

$$\text{Lipid yield (\%)} = [\text{Lipid mass (g)} / \text{Dry biomass (g)}] \times 100 \quad (4)$$

$$\text{Lipid productivity (g/L/day)} = \text{Biomass productivity (g/L/day)} \times \text{Lipid yield (\%)} \quad (5)$$

Results and Discussion

Isolation and Identification of Microalgae

Microalgae were collected and isolated from four marine and three freshwater locations covering offshore areas, coastlines, and inland water bodies within Sabah, Malaysia. These locations were categorised as either marine or freshwater based on their distinct geographical features and the salinity (ppt) of the water bodies, as recorded in Table 1. The collected samples were enriched with the appropriate culture media according to the source and corresponding salinity. Following isolation, the microalgae species were maintained using the same media throughout the cultivation period. However, not all collected strains were able to thrive in the medium. This may have been due to the specific nutrient requirements of each of the different species, nutrient imbalances, salinity stresses, unsuitable pH levels, or the presence of contaminants, which hindered growth (Panahi *et al*., 2019).

To optimise growth and biomass productivity, the preparation of different types and compositions of media, adjusted for salinity, nutrient levels, and pH, may be needed depending on the specific requirements of each isolate. Additionally, these microalgae may exhibit different trophic modes such as autotrophy, heterotrophy, or a preference for mixotrophic conditions (Abreu *et al*., 2022), which affect their light absorption and nutrient assimilation capacities. Therefore, the composition and conditions of the growth medium have a significant impact on the adaptability, growth, and successful culture establishment of microalgae isolated from natural waters.

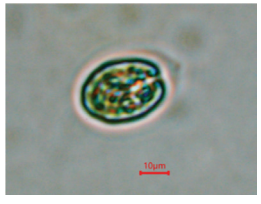
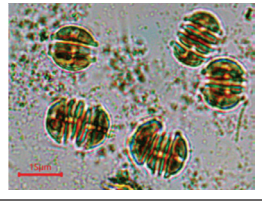
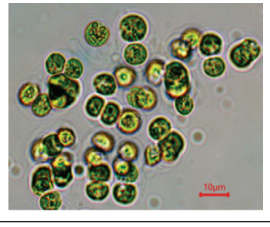
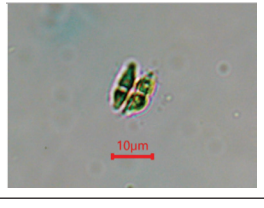
The summary of sampling location details and microscopic images of isolates are illustrated and recorded in Table 1. The morphological characteristics of each isolate are also described. Based on the query cover and percentage identity of sequence searches, it was found that the marine microalgal isolates were closely related to *Tetraselmis striata* (MM3=99.86%, MM4=99.10%), *Caecitellus pseudoparvulus* (99.69%), *Caecitellus parvulus* (95.45%), and *Halamphora subtropica* (97.30%) while freshwater isolates were closely related to *Mychonastes afer* (95.42%), *Nitzschia hantanense* (99.88%), *Monactinus sturmii* (100.00%), and *Pseudomuriella* sp. (95.08%).

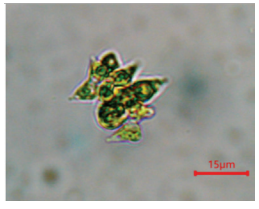
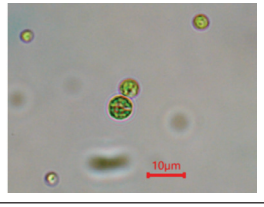
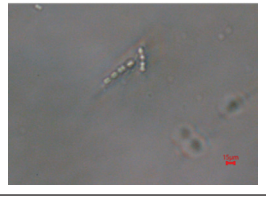
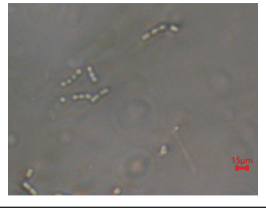
Samples MM1 and MM2, both belonging to the genus *Caecitellus*, exhibited very similar morphological features despite being different species. Likewise, MM3 and MM4, although isolated from two different locations, belonged to the same species (*Tetraselmis striata*) and therefore displayed identical or highly similar morphological characteristics. Based on the morphological observation (Table 1), the marine isolates exhibited greater structural diversity compared with the freshwater isolates.

Marine species such as *Caecitellus parvulus* and *C. pseudoparvulus* were characterised by small, round green cells arranged in S-shaped chains while *Tetraselmis striata* displayed motile, elliptical cells with distinct flagellar emergence points, and *Halamphora subtropica* showed a semi-lanceolate form with curved and truncated ends.

In contrast, the freshwater isolates were comparatively simpler in morphology, dominated by small round or lanceolate cells (*Mychonastes afer*, *Nitzschia hantanense, Pseudomuriella* sp.), with the exception of *Monactinus sturmii*, which formed elaborate colonies arranged in flat concentric rings. This suggests that marine isolates display greater structural diversity because the marine environment is more heterogeneous and dynamic characterised by varying light penetration, salinity, turbulence, and nutrient availability (Soccodato *et al*., 2014).

Table 1: Sampling location and microalgae isolates details summary including morphological observations

Source	Isolate	Species	Sampling Location Details	Microscopic Image	Media	Morphological Description
Marine	MM1	*Caecitellus parvulus*	Likas Bridge (river) 6.02756° N, 116.11956°E		Walne's Media	Small green, round cells often arranged in S-shaped chains.
	MM2	*Caecitellus pseudoparvulus*	Sungai Salut (coastal) 6°01'09.7"N116°07'06.2"E		Walne's Media	Small green, round cells often arranged in S-shaped chains.
	MM3	*Tetraselmis striata* (Salut)			Walne's Media	A motile, green elliptical cell with an apical depression where flagella emerge from.
	MM4	*Tetraselmis striata* (Likas)	Likas (coastal) 6°00'54.4"N116°06'52.5"E		Walne's Media	A motile, green elliptical cell with apical depression where flagella emerge from.

	MM5	*Halamphora subtropica*	Kingfisher (estuary) 6°01'09.7"N116°07'06.2"E		Walne's Media	Semi-lanceolate cell with a curved dorsal side and truncated ends.
Freshwater	FM1	*Mychonastes afer*	Nexus Karambunai (pond) 6°06'55.3"N116°06'24.1"E		Bold's Basal Media	Motile, round green cell
	FM2	*Nitzschia hantanense*			Walne's Media	Lanceolate cell with a narrow centre and rounded ends.
	FM3	*Monactinus sturmii*	Bandana (pond) 5.4413390°N116.1612334°E		Bold's Basal Media	Cells are arranged in flat concentric rings to form colonies (coenobia). Individual cells are cone shaped.
	FM4	*Pseudomuriella* sp.	Kolam Greenview (pond) 5.451766° N116.234552° E		Bold's Basal Media	Small, round green cell.

Growth Characteristics and Growth Rates

Overall, all nine isolates exhibited varying growth patterns, although most followed the typical microalgal growth curve consisting of the lag phase, exponential phase, stationary phase, and death phase (Azmi *et al*., 2018) over a 25-day period, as shown in Figure 1. Most isolates experienced short lag phases of up to three days, except for isolates FM3 (*M. sturmii*) and FM4 (*Pseudomuriella* sp.), which exhibited prolonged lag phases of up to six days.

Among the marine isolates, *T. striata*, MM4, and MM3 exhibited extended exponential phase lasting up to 15 and 18 days, respectively. *T. striata* 2 from Salut exhibited the highest cell density (178.8 x 10^4 cells/mL) and a high growth rate (0.26 day^{-1}), as shown in Table 2. By comparison, *C. pseudoparvulus* had the shortest exponential phase, reaching up to day 6. According to Patidar *et al*. (2018), the growth rate achieved by *T. striata* was higher than that of this study with a specific growth rate of 0.34 day^{-1} when cultivated in modified algal growth medium in photobioreactor.

Furthermore, *M. afer* had the shortest exponential phase, up to day 10 while *Pseudomuriella* sp., had the longest, which was up to 21 days, among the freshwater isolates. Notably, *M. afer* recorded the highest cell density (271.3 x 10^4 cells/mL) and growth rate (0.37 day^{-1}), followed by isolate *Pseudomuriella* sp., with a cell density and growth rate of 239.8 x 10^4 cells/mL and 0.23 day^{-1}, respectively. No specific growth rate (μ) of *M. afer* was reported in literature.

However, it was reported to have a doubling time of 12.9 hours in a study by Yuan *et al*. (2011) with *M. afer* HSO-3-1 strain. The differences of growth obtained may be attributed to species-specific intrinsic growth rate (Hiraoka *et al*., 2020), suggesting that strains with shorter exponential phases and higher population densities naturally undergo more rapid cell division and population doubling. Otherwise, a short exponential phase could also indicate rapid depletion of nutrients in the growth medium, increased competition among cells, and an accumulation of toxic metabolic by-products, which collectively slow down growth and initiate the transition to the next phase (Azmi *et al*., 2018). During the stationary phase, isolates *M. sturmii*, *C. pseudoparvulus*, and *H. subtropica* maintained a steady growth for six to seven days while *C. parvulus* and *N. hantanense* exhibited shorter stationary phase lasting around three days.

Furthermore, *M. afer* and *T. striata* (MM4) appeared to have a shorter stationary phase, which may have occurred during intervals when cell density measurements were not recorded. During this phase, growth of microalgal has slows as the rate of cell division becomes equal to the rate of cell death. In contrast, a prolonged and stable stationary phase observed in other isolates may indicate that certain species possessing greater adaptability and survival mechanisms.

These mechanisms allow cells to endure increasingly stressful conditions in the growth medium, pH fluctuations, depleting nutrients, and accumulation of metabolic waste. Adaptations may include metabolic shifts that reprogram cellular metabolism to conserve energy, reducing growth-related processes and redirecting carbon flux toward the synthesis of reserve compounds (Abdul-Sani *et al*., 2024). The accumulation of these compounds including carbohydrates and neutral lipids supports extended viability, thereby maintaining a stable population over time.

As cultures transitioned into the death phase, cell densities declined at different rates across isolates, generally beginning on day 15 or day 18. Notably, FM4 exhibited a delayed decline, with cell density decreasing only after day 20. This phase reflects inhibited cell division and the exhaustion of essential nutrients needed for continued survival. Interestingly, *T. striata* from different locations exhibited different growth patterns, shown in Figure 1A. These differences may reflect local adaptations to environmental variations, including light intensity, nutrient availability, and salinity in their respective native habitats.

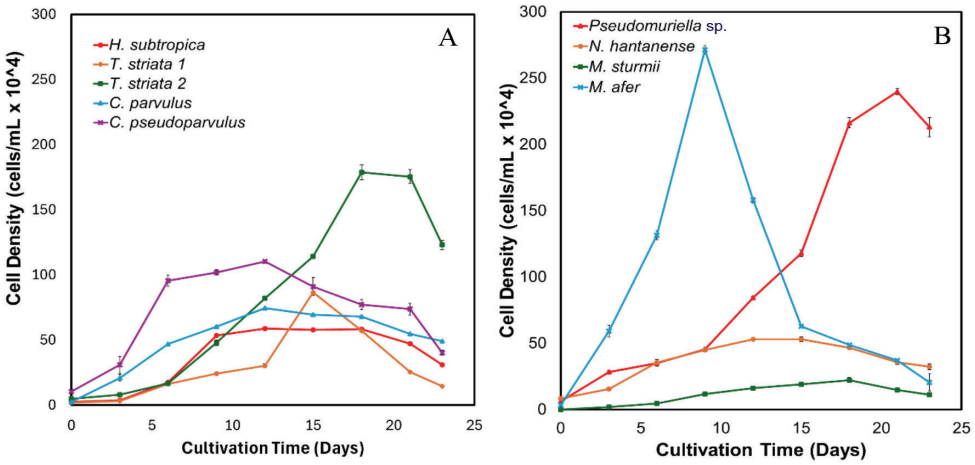


Figure 1: Growth curves of isolates from marine (A) and freshwater (B) sources presented as mean cell density against cultivation time

Biomass Productivity, Lipid Yield, and Productivity

Biomass productivity, lipid content, and lipid productivity of the nine isolates are shown in Table 1, Table 2, and lipid content as percentages to biomass dry weight (%DW) illustrated in Figure 4. Marine and freshwater microalgal species produced varying amounts of biomass and lipid yield at different phases of growth.

However, marine isolates generally produced higher dry biomass weight compared to freshwater isolates during both the exponential and stationary phases. Biomass productivity was higher during the exponential phase but decreased as cultures transitioned into the stationary phase for freshwater isolates but varies for marine isolates as biomass productivity for *C. parvulus* and *H. subtropica* increased during its stationary phase. Among marine isolates, *C. pseudoparvulus* had the greatest biomass productivity at 6.57 mg/L/day, followed by *T. striata* (MM3) at 3.83 mg/L/day during the exponential phase. During the stationary phase, *C. parvulus* recorded the highest biomass productivity of 5.05 mg/L/day, followed by *H. subtropica* with 4.06 mg/L/day. For freshwater isolates, *N. hantanense* achieved the highest biomass productivity at 4.17 mg/L/

Table 2: Comparison of specific growth rate (μ) of each isolate between marine and freshwater species

Sample	Species	Specific Growth Rate, μ (day^{-1})
FM1	*Mychonastes afer*	0.37
FM2	*Nitzschia hantanense*	0.12
FM3	*Monactinus sturmii*	0.12
FM4	*Pseudomuriella sp.*	0.23
MM1	*Caecitellus parvulus*	0.28
MM2	*Caecitellus pseudoparvulus*	0.23
MM3	*Tetraselmis striata* 1	0.25
MM4	*Tetraselmis striata* 2	0.26
MM5	*Halamphora subtropica*	0.24

day during the exponential phase and 3.88 mg/L/day during the stationary phase.

Microalgae biomass increased after the cells adapted to the culture medium, rapidly consuming nutrients for growth and cell division during exponential phase, thereby maximising growth and biomass production (Ramlee *et al*., 2021). Nitrogen, a key macronutrient plays a crucial role in enhancing biomass production as it is essential for protein biosynthesis and various cellular functions (Yaakob *et al*., 2021). The biomass production of each species varies, indicating that they have different capacities of assimilating nitrogen and sensitivities to environmental conditions such as light absorption, resulting in these differences in biomass production for the same culture medium and culture conditions.

Despite Bold's Basal Media in the medium having higher nitrate levels (0.25g/L) than Walne's Media (0.1g/L), marine isolates managed to have a higher nitrogen utilisation and removal capacity for biomass production (Zhang *et al*., 2023), which may have also been influenced by their sensitivity towards the salinity levels of the seawater in the culture medium (Pan *et al*., 2024).

Besides that, the higher nitrate concentrations in BBM have contributed to higher growth rate and maximum cell density of freshwater isolates based on Figure 3. The decrease in biomass productivity of some isolates indicates that biomass production decreased as nutrients in the culture medium become the limiting factor (Panahi *et al*., 2019), thereby slowing down microalgae growth as nutrients depleted during the stationary phase (Figure 4).

The lipid content and productivity of isolates vary for marine and freshwater species as shown in Figure 2 and Figure 3. Some isolates showed an increase or decrease of lipid content from exponential to stationary phase, having different responses and adaptations to the increasing stress conditions in the growth medium throughout the period of cultivation. For marine isolates, *C. parvulus* showed the highest lipid content at 54.6%, followed by *C. pseudoparvulus* at 28.4% with lipid productivities of 193.85 and 186.56 mg/L/day, respectively during the exponential phase. Lipid studies of *C. parvulus* have not been reported in any literature, making this species a potential oleaginous candidate species to be further explored due to the high lipid yield obtained.

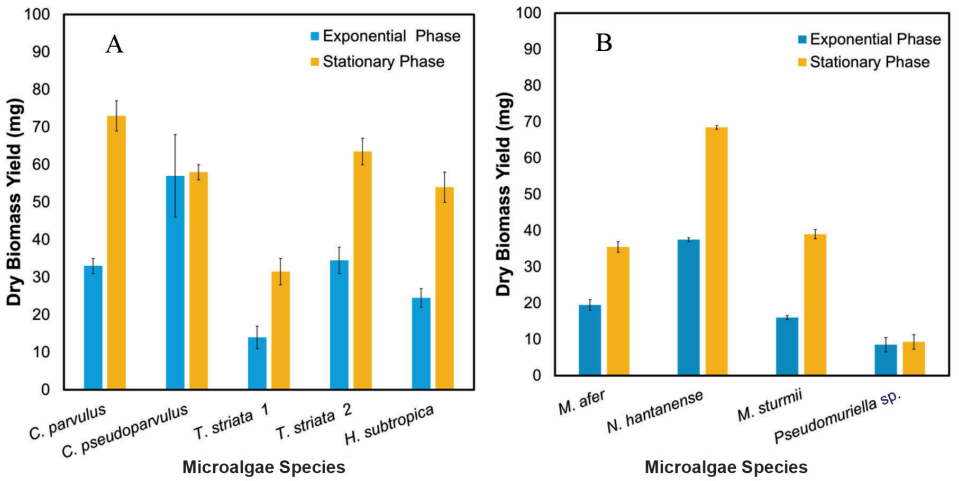


Figure 2: Comparison of Dry Biomass Weight (DW) of each isolate between marine (A) and freshwater species (B) during exponential and stationary phases. All data are represented as the means ± standard deviation

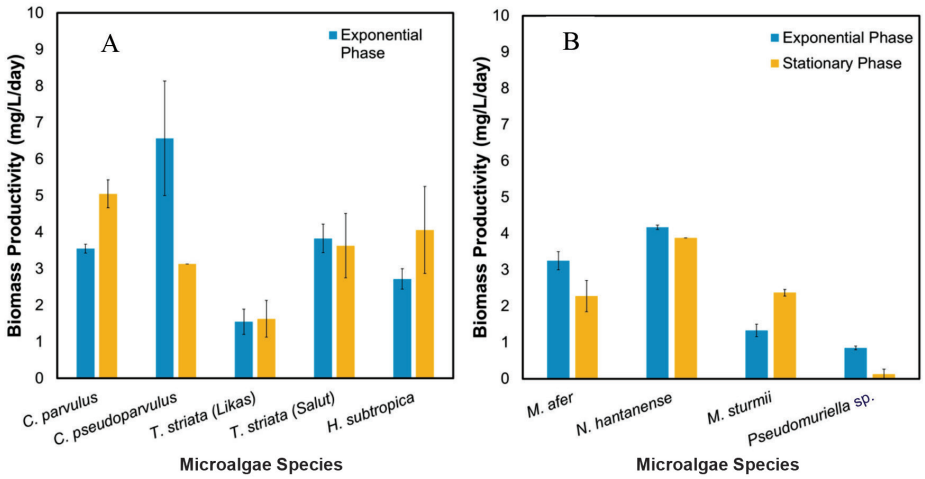


Figure 3: Comparison of biomass productivity (mg/L/day) between marine (A) and freshwater (B) isolates during exponential and stationary phases. All data are represented as the means ± standard deviation

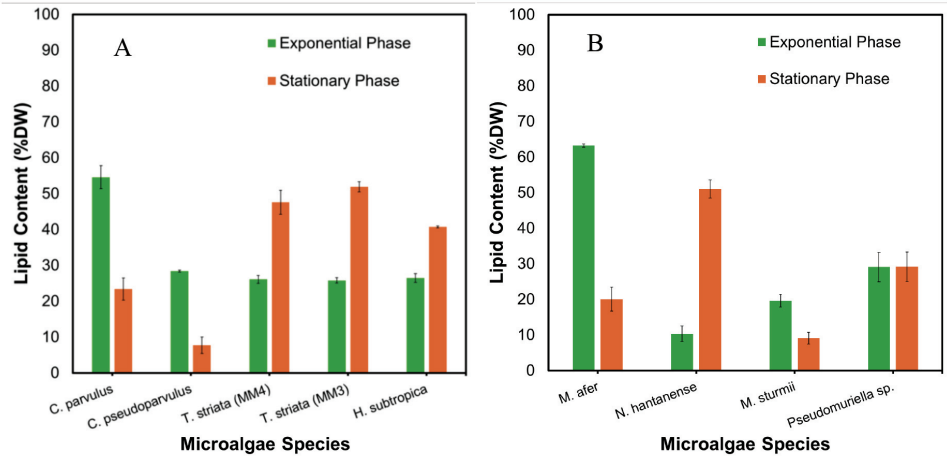


Figure 4: Comparison of lipid content (%DW) between marine (A) and freshwater (B) isolates during exponential and stationary phases. All data are represented as the means ± standard deviation

During the stationary phase, lipid content and productivity of marine isolates increased except for *C. parvulus* and *C. pseudoparvulus*. The highest lipid content and productivity obtained was 51.9% and 188.4 mg/L/day by *T. striata* (MM3) in this phase. According to literature, the lipid yield obtained from *T. striata* ranges between 23.3% (Patrinou *et al.*, 2023) and 43.2% (Monteiro *et al.*, 2023) while the range for *H. subtropica* was between 25% and 42.87% (Vello *et al.*, 2023). The lipid yield for *H. subtropica* was 40.7%, which falls within this range, thus, supports the findings of this study.

Whereas *M. afer* showed the highest lipid content of 63.25% and lipid productivity of 205.57 mg/L/day for freshwater isolates in the exponential phase. Meanwhile, *N. hantanense* produced the highest lipid content and had high lipid productivity of 51.05% and 180.30 mg/L/day, respectively during stationary phase. The lipid content of *Pseudomuriella* sp. remained relatively constant in both phases of growth

however, lipid productivity decreased from 24.8 to 3.64 mg/L/day.

According to Yuan *et al*. (2011), *M. afer* HSO-3-1 yielded lipid content of 53.9% DCW, which was lower than the lipid yield obtained in this study (63.25%). Additionally, lipid yield obtained by *Nitzschia* sp. were 46.11% (Kholssi *et al*., 2023) but could reach up to 51.2% under salinity stress (Cheng *et al*., 2014) in which the lipid yield of *N. hantanense* (51.05%) obtained falls within this range.

It is possible that lower amounts of lipids were extracted due to the challenges prior and during lipid extraction. Pre-treatment of the biomass obtained may be necessary to disrupt the tough cell walls and release lipids, facilitating the lipid recovery process (Kamdem *et al*., 2023). Besides that, during solvent extraction, increasing the chloroform:methanol:water ratio to 2:2:1.8 (Gorgich *et al*., 2020) may improve the extraction and efficiency of the solvent by dissolving more lipids into the chloroform, thus, increasing the lipid content.

Generally speaking, lipid accumulation occurs in the stationary phase to prolong the survival of the cell (Abdul-Sani *et al*., 2024). However, not all microalgae accumulate lipids to the same extent under the same conditions. Some microalgae species tend to accumulate carbohydrates more than lipids for survival under stress conditions instead (López-Pacheco *et al*., 2023), which may suggest the decrease in lipid content was due to competition for carbon resources (Figure 5).

Conclusions

The characterisation of growth features, determination of biomass productivity, lipid content, and lipid productivity were performed for all nine isolates. All nine isolates showed different growth rates, biomass productivity, and lipid content in both exponential and stationary phases. For marine species, *C. parvulus* showed high lipid content and productivity of 54.6% and 186.56 mg/L/day, respectively during exponential phase. For freshwater species, *M. afer* showed the highest cell density (271.3 x 10^4 cells/mL) and growth rate (0.37 day^{-1}), followed by high lipid content (63.25%) and lipid productivity (205.57 mg/L/day) during exponential phase.

These findings suggest that *C. parvulus* and *M. afer*, each from marine and freshwater sources are potentially valuable species for biodiesel production. Further studies on the optimisation and enhancement of their lipid production may be required for these two selected species. This study contributes to the expansion of knowledge on marine and freshwater microalgae species that have the potential to be commercially viable as a source of renewable energy and other biotechnology products.

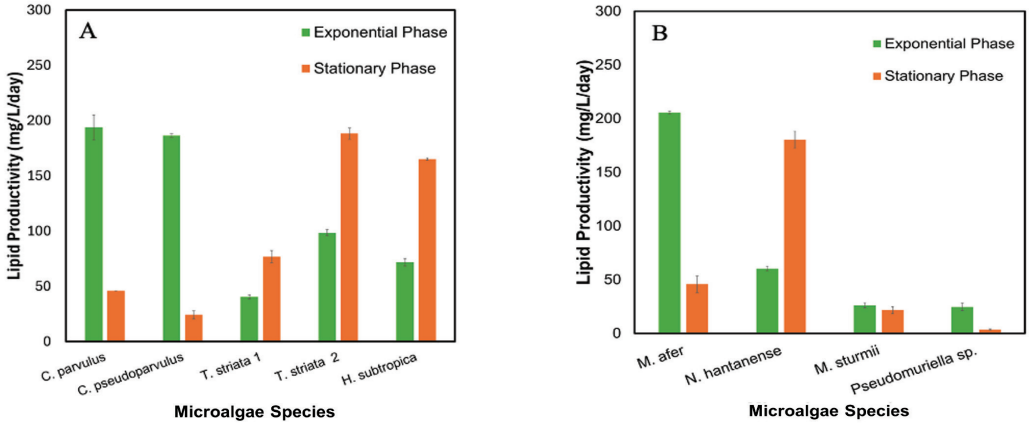


Figure 5: Lipid productivity (mg/L/day) of marine (A) and freshwater (B) isolates at exponential phase and stationary phases. All data are represented as the means ± standard deviation

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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