Original Article

Isolation, culture trials, and biochemical composition of microalga *Tetraselmis* from coastal waters of Tanzania

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Abstract

Microalgae are primary producers in aquatic ecosystems. Their high nutritional value make them suitable for applications in aquaculture and biotechnology. Serial dilution techniques were used to isolate the green microalga *Tetraselmis* sp. from water samples collected from the Ruvu Estuary in Tanzania. Laboratory culture trials were undertaken at varying salinity and light intensity levels, followed by biochemical analysis. Intermediate salinities (15 and 35) favoured cell accumulation, and light intensity significantly influenced *Tetraselmis* biochemical composition. Low light intensity (2.9 ± 1.6 Klux) and early harvests (day 7) increased the protein, lipid, carbohydrate and fibre content, whereas high light intensity (5.5 ± 3.3 Klux) led to greater ash accumulation. The day 16 harvest contained higher levels of minerals, with calcium, potassium, and magnesium being prominent. Trace minerals, including selenium, were present in safe quantities. The potential of *Tetraselmis* sp. from coastal Tanzania for aquaculture and biotechnological applications is highlighted.

Keywords: microalgae, *Tetraselmis* sp., Tanzania coastal waters, growth conditions, biochemical composition

Introduction

Microalgae are primary producers and play an important role in aquatic ecosystems as they support food webs. Microalgae have gained increasing attention for their potential applications in aquaculture, biotechnology, and environmental restoration. Due to their high-quality nutritional profile, they are extensively cultured and utilized as natural food sources in hatcheries for the rearing of fish, molluscs, and crustacean larvae (Brown, 2002; Sirakov *et al.*, 2015). These natural foods improve the growth, health and survival of fish and shellfish larvae. However, the nutritional value and growth can be influenced by various factors, including light intensity, nutrient limitation, salinity, temperature, and pH (Creswell, 2010). Among these factors, salinity and light are particularly emphasized as primary factors for marine species, as they can be adjusted and maintained at desirable levels without causing adverse effect on the cultured organism (Hotos and Avramidou, 2021). Salinity affects growth of microalgae through acting directly on the osmoregulation process. Although all marine species can survive in wide ranges of salinity, high salinity can inhibit the growth and alter chemical composition of some species (Khatoon *et al.*, 2018). Likewise, light is paramount and a critical factor to microalgae as it directly affects photosynthesis from which growth and biomass production is ensured. Depending on microalgae species, light intensity increases growth rate, but extreme intensity up to saturation point may lead to photo-inhibition (Metsoviti *et al.*, 2019).

Despite the pivotal role that microalgae play in marine ecosystems and their potential industrial applications, a significant gap exists in the knowledge of the microalgal composition within the coastal waters of Tanzania. The microalgae *Tetraselmis*, known for their diverse biochemical composition and potential use

in aquaculture and biotechnology, remain largely un-investigated in this region. Limited studies have explored the aquaculture potential of microalgae in Tanzania, with a focus on specific species such as the cyanobacteria *Arthrospira fusiformis* in the Momella Lakes (Michael *et al.*, 2019; Mulokozi *et al.*, 2019), and a freshwater green algae *Chlorella vulgaris* (Mtaki *et al.*, 2021). However, comprehensive investigations into the cultivation and nutritional profiles of uni-algal marine species in the Western Indian Ocean remain notably limited. Most of the available research has concentrated on the distribution, composition and abundance of microalgae in this region (Kyewalyanga, 2002; Moto *et al.*, 2018; Sekadende *et al.*, 2021). With the growing demand for seafood and increasing attention towards the biotechnological applications of microalgae, there is a need to explore potential microalgae species for aquaculture and other industrial uses. This study addresses the pressing need for comprehensive research on *Tetraselmis* sp. in Tanzanian coastal waters by aiming to achieve three critical objectives: isolate *Tetraselmis* strains from the local environment; conduct laboratory culture trials to assess growth in terms of cell density in response to variations in salinity and light intensity; and analyse the biochemical composition of the isolated strain.

Materials and methods

Water sampling and microalgae isolation

Water sampling for microalgae isolation was carried out along the gradient of the Ruvu Estuary, Bagamoyo, Tanzania (6.384°S, 38.86°E) during both the Southeast monsoon and Northeast monsoon periods to increase the chance of encountering the microalgae with aquaculture potential. Samples were collected at brackish and full-strength salinity stations using a 10 litre bucket. Three sampling points were established at each sampling station. The *Tetraselmis* strain (Fig. 1B) used in the current study was obtained during the Southeast monsoon. At each sampling point, 100 litres of water were concentrated through a plankton net with 20 µm mesh size, and these samples were then combined to create one sample. The concentrated crude samples were transferred into glass bottles, and immediately enriched with a few drops of the standard laboratory culture medium, Guillard's F/2 (Kang *et al.*, 2011). The crude sample was exposed to ambient light near the windows for five days, with gentle mixing in the laboratory at the University of Dodoma. This technique was primarily conducted to exclude certain microorganisms with limited mobility. During this time, the flagellated algae concentrated themselves on one side of the flask in response to the light, making it easy to collect them using a pipette. The motile algae were then transferred into other flasks where the serial dilution technique with Guillard's F/2 medium was applied until the uni-algae strain of *Tetraselmis* sp. was obtained. Using the serial dilution technique, ten test tubes, each containing 9 ml of the culture medium, were prepared. After incubation for two days, 1 ml of the microalgae culture from one test tube was transferred to another test tube containing fresh culture medium. This transfer process was continuously repeated accompanied by regular microscopic checks. To ensure aseptic conditions, all flasks and the culture media used in the isolation process were sterilized before use. The identification of the isolated *Tetraselmis* strain was based on morphological features observed under a compound light microscope.

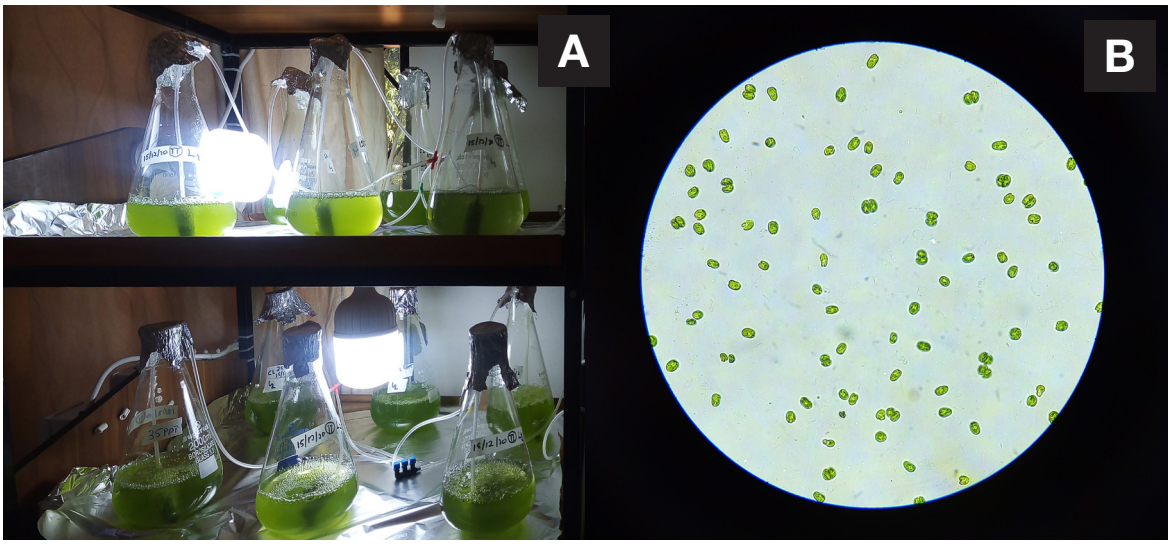

Figure 1. Laboratory set up for *Tetraselmis* sp. culture (A). Cells of *Tetraselmis* sp. under microscope, at magnification of 100X (B).

Experimental design

The successfully isolated strain of *Tetraselmis* sp. (Fig. 1B) was tested for growth, and accumulation of biochemical composition under different levels of salinity and light intensty to determine the optimal laboratory culture conditions. Three different experiments were carried out, with the first two lasting for 14 days each and the third extending to 16 days. The initial experiment focused on evaluating the effect of different light intensities on *Tetraselmis* sp. cell density at a salinity level of 35, reflecting the conditions in which the strain was originally collected from full-strength salinity water. Experiment 2 aimed to assess how different salinity levels influence the accumulation of *Tetraselmis* sp. cell density under top-light conditions. The final experiment analysed the biochemical composition of a microalga cultured under varying light intensities while maintaining a constant salinity of 35. Commencing each experiment involved obtaining a sub-culture sample from the mother culture and inoculating it with a fresh culture medium of Guillard's F/2 medium. The microalgae culture (100 ml) was inoculated with 1900 ml of the culture medium in an Erlenmeyer flask and placed in the growth chamber (Fig. 1A) at a temperature of 28-29 °C while being gently aerated. The culture was illuminated with artificial light provided by white LED lamps at a photoperiod of 12/12 hours for day and night.

Assessment of growth performance under varying light intensity and salinity levels

To evaluate the effect of varying light intensity on the growth of *Tetraselmis* sp., the culture was illuminated by the artificial light positioned either at the top or on the side of the culture flasks. The light was quantified by a VXLM_636 Vertex light meter. The top light supplied an average intensity of 5.5 ± 3.3 Klux while the side light supplied an intensity of 2.9 ± 1.6 Klux to the culture. For testing the effect of salinity on the growth of *Tetraselmis* sp., five levels of salinities i.e. 5, 10, 15, 35 and 40 were set under the top light. Different salinities used in the testing of growth were prepared by either diluting the ocean water, or in the case of preparing the salinity of 40, table salt was added to the seawater. The growth was determined in terms of cell density which was counted daily using a haemocytometer under a compound microscope (Olympus, BM 190, Japan). One millilitre of a sample was taken from the main culture, fixed with lugol solution and placed on the haemocytometer for observation and counting at a magnification of 100 X.

Assessment of biochemical composition of *Tetraselmis* sp.

The experiment was conducted under both top-light and side-light laboratory conditions, with light intensities ranging from 5.5 ± 3.3 Klux to 2.9 ± 1.6 Klux, respectively. Biomass for analysing the biochemical composition of *Tetraselmis* sp. was obtained by harvesting the culture on day 7 and 16 of the experiment. This strategy aimed to examine potential shifts in the biochemical composition of *Tetraselmis* sp. across different stages of its growth cycle. At day 7, a subsample (100 ml) was taken from the main culture while at the end of experiment the entire culture was harvested. The harvested sample was left to settle for about two hours and the supernatant was decanted and discarded. The concentrate was then centrifuged (Model FC5706, Ohaus Corporation, Germany) for 5 minutes at 4430 rpm to obtain the wet biomass. The obtained biomass was spread thinly on the plates then placed in the oven for 2 hours to dry to constant weight at a temperature of 105 °C. The dry biomass was then ground to powder and kept in a cool place until required for the analysis of proximate composition and mineral contents.

The proximate analysis of crude protein, lipid, fibre, ash and soluble carbohydrates in the *Tetraselmis* sp. biomass was conducted following standard analytical methods. The crude protein was determined using the semi-micro Kjeldahl digestion and indophenol blue colorimetric methods (Emteryd, 1989; Quarmby and Allen, 1989). On the other hand, lipid content was analysed according to Bligh and Dyer (1959). This method involved the addition of the mixture of three organic solvents (chloroform, methanol, and water) in specific ratios to the polyethylene vial containing 2 g biomass of *Tetraselmis* sp. After the extraction, the filtrate was transferred into a graduated separating funnel where the upper alcoholic layer of methanol and water was discarded while the mixture of chloroform and lipid was retained. The chloroform was then evaporated under vacuum by using rotavapor (Heidolph, Germany) at a temperature of 40 °C, and the resulting residue, which contained lipids, was oven-dried for three hours at 50 °C followed by weighing to determine the percentage lipid composition.

The crude fibre content was determined gravimetrically after acid-alkali hydrolysis (Allen, 1989; Quarmby and Allen, 1989). In this method, 1 g of *Tetraselmis* sp. powder was added into boiling sulphuric acid (1.25 % v/v) then washed with boiling water. The extracted sample

was added to 1.25 % sodium hydroxide for further extraction and then washed with boiling water while suction pumping to separate it from the alkali. After oven drying the extracts at 105 °C for 2 hours, the sample was weighed followed by calculation to obtain the composition of fibre in the biomass. Total soluble carbohydrate of the *Tetraselmis* sp. biomass was extracted by hot water and estimated by calorimetric procedures after reacting with anthrone reagent (Allen, 1989).

The total mineral content of the biomass, termed as ash, was determined by combusting 1 g of the sample at 550 °C in the muffle furnace for 2 hours. The ash was then weighed and the percentage composition calculated. The determination of the concentration of individual mineral in the biomass followed the procedures described by Emteryd (1989). The dry sample (0.1 g) was oxidized using a mixture of acids (1 ml per-chloric acid and 5 ml nitric acid) followed by reading the concentration in the Atomic Absorption Spectrophotometer (AA240 Varian, USA). The analysed minerals were the macro elements calcium, magnesium, potassium and phosphorus, and the two trace minerals iron and selenium (antioxidant).

Data analysis

All collected data were analysed using SPSS software (IBM SPSS, version 20) while the graphs were drawn using R software (version 4.03). The variation in the growth performance under different conditions and biochemical compositions in *Tetraselmis* sp. biomass was analysed using the general linear model (GLM). Tukey Honest Significant Difference (THSD) test was used for multiple comparisons of salinity means when a significant difference existed between the salinity levels. A p-value of less than 0.05 was considered significant.

Results

Effect of light on cell density

Tetraselmis sp. showed an exponential growth trend in both light orientations (Fig. 2). There was no statistical variation between the two lights ($F = 1.093$; $p = 0.301$) although the top light seemed to surpass the side light. However, significant variation was observed among the culture time ($F = 11.103$; $p < 0.05$) whereas the organism accumulated more cell density as time went on. The maximum cell density for both of the light orientations was recorded at day 12 with the density reaching $4.5 \pm 0.728 \times 10^6$ cells/ml for top light and $3.5 \pm 2.133 \times 10^6$ cells/ml for side light. The only culture which entered a stationary phase was that of side light from day 12 to 14, while the culture with top light orientation did not display that trend.

Influence of salinity on cell density

The growth of *Tetraselmis* sp. was slow for all salinity levels in the first three days of the experiment as the organism was adapting to the culture environment (Fig. 3). After the take-off, a good growth trend was observed in the salinity of 25 and 35 while poor growth was observed in the extreme salinity levels of 5 and 40. The microalgae continued to grow without showing either stationary or death phases for the

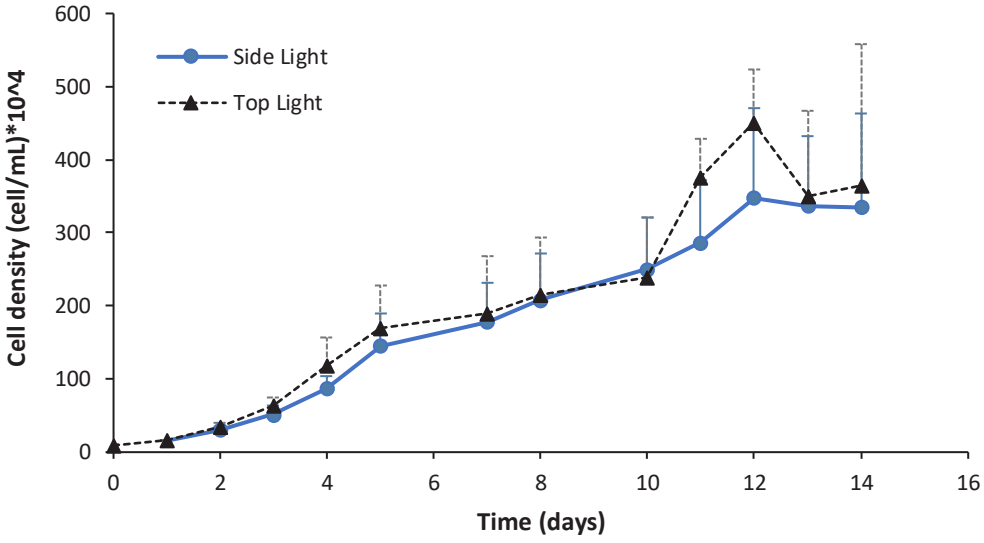

Figure 2. Growth performance of *Tetraselmis* sp. (± SD) at different light orientations during the culture experiment (days).

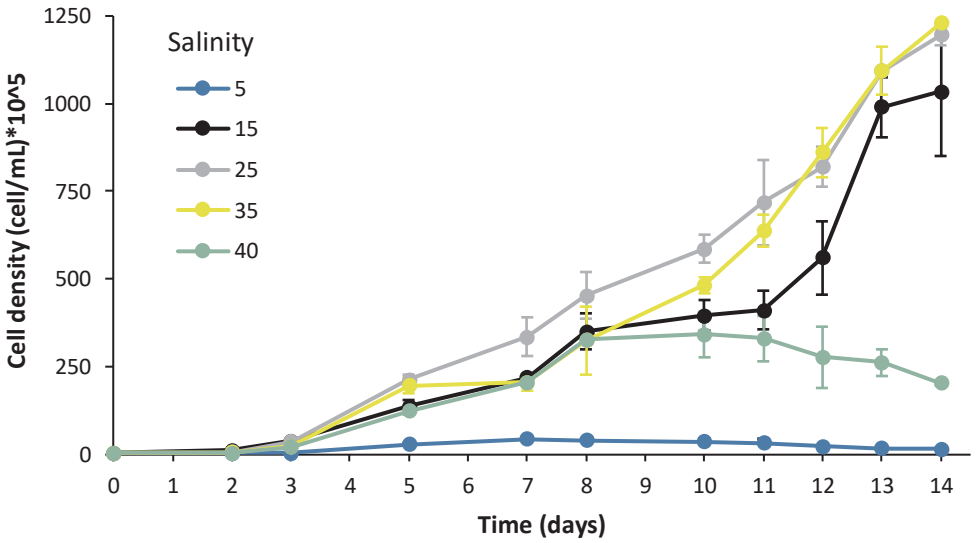

Figure 3. Growth performance of *Tetraselmis* sp. (± SD) at different salinity levels during the culture experiment (days).

salinities of 15 to 35. There was a significant difference in cell densities between salinity levels ($F = 371.511$; $p < 0.05$). The Tukey multiple comparisons test revealed significant variations in cell density between all salinity levels except the salinity of 25 and 35 (Table 1). The highest mean cell density (1230.5 ± 2.263 x10^5 cells/ml) was attained at day 14 in a salinity of 35, while on the same day salinity 5 attained the minimum density (15.1±1.555 x10^5 cells/ml). There was also a significant effect of culture time ($F = 294.241$; $p < 0.05$) and combined effect of salinity and time ($F = 38.203$; $p < 0.05$) on the growth of *Tetraselmis* sp. Exponential growth of the organism occurred for all salinity levels from day 3 except salinity 5 which behaved differently, and salinity 40 which showed a decline in cell density from day 11 of the experiment.

Influence of light on biochemical composition

There was a significant effect of light intensity ($F = 91.29$; $p < 0.05$), harvesting day ($F = 188.83$; $p < 0.05$) and interactive effect of light intensity and harvesting day on the protein content of *Tetraselmis* sp. There was a high

Table 1. Tukey HSD multiple comparisons of cell density between salinity levels.

Salinity	Salinity	Mean Difference Cells/ml	P-value
5	15	-355*	< 0.001
	25	-474*	< 0.001
	35	-438*	< 0.001
	40	-168*	< 0.001
15	5	355*	< 0.001
	25	-119*	< 0.001
	35	-83*	< 0.001
	40	187*	< 0.001
25	5	474*	< 0.001
	15	119*	< 0.001
	35	36	0.116
	40	305*	< 0.001
35	5	438*	< 0.001
	15	83*	< 0.001
	25	-36	< 0.001
	40	269*	< 0.001
40	5	168*	< 0.001
	15	-187*	< 0.001
	25	-305*	< 0.001
	35	-269*	< 0.001

Table 2. Comparative total mean of proximate composition between the light intensities.

Composition (%)	Light intensity		P-value
	2.9 ± 1.6 Klux	5.5 ± 3.3 Klux	
Crude protein	67.91 ± 2.27	64.67 ± 7.31	P < 0.05
Crude lipid	11.16 ± 1.25	9.38 ± 0.57	P = 0.941
Carbohydrate	5.95 ± 0.24	5.45 ± 1.16	P = 0.402
Ash	2.73 ± 0.18	6.65 ± 2.17	P = 0.010
Crude fibre	1.34 ± 0.05	1.25 ± 0.12	P = 0.830

average protein content (67.91 ± 2.27 %) in the biomass cultivated in low light intensity compared to 64.67 ± 7.31 % recorded in high light intensity (Table 2). Similarly, the biomass accumulated a large quantity of protein on day 7 of growth compared to day 16 (Table 3).

The composition of crude lipid, carbohydrate, fibre and ash was also affected significantly by light intensity, harvesting time and the interactive effect of the two parameters. In a similar scenario to protein, a high quantity of crude lipid, carbohydrate, and fibre was recorded in the biomass cultivated with low light intensity (Table 2), harvested on day 7 (Table 3). The content of ash was contrary to other proximate components where a high average quantity (6.65 ± 2.17 %) was recorded in the high light intensity, and in the biomass harvested at day 16 (8.63 ± 2.17) of the culture experiment.

The contents of all quantified minerals except phosphorus were significantly high in the biomass harvested on day 16 (Table 4). The biomass harvested on day 16 was composed of a large quantity of calcium, followed by potassium and magnesium while the tracer minerals were in lower quantities. Comparing the influence of light, *Tetraselmis* sp. accumulated more mineral content when cultivated with high light intensity, whereas calcium contributed the highest amount (Table 4). On the other hand, the mineral phosphorus was abundant in the biomass cultivated at the low light intensity, although it was preceded by calcium.

Discussion

Tetraselmis sp. is a marine microalga widely used in aquaculture as a natural food for fish and shellfish larvae due to its valued nutritional content and ease of culture under hatchery conditions (Meseck *et al.*, 2005). In this study, *Tetraselmis* sp. exhibited optimal growth in intermediate salinities of 15 and 35, while extreme salinities of 5 and 40 led to comparatively poor growth. This trend indicates *Tetraselmis*' sp. preference for moderate salinity ranges, which is in line with the requirements of many marine species. Salinity variation influences microalgae growth performance, physiological as well as biochemical processes (Pal *et al*., 2010). Depending on species, salinity causes stress to microalgae when it is above the survival range and may have significant effects on the growth and other body processes (Pandit *et al*., 2017). Despite originating from a marine environment, *Tetraselmis* sp. exhibited poor growth in a salinity of 40 under laboratory conditions, deviating from previous findings (Pugkaew *et al*., 2019; Hotos and Avramidou, 2021) that reported good performance even at salinity above 40. This variation in findings may be linked to differences in the strain used and the species' adaptation to its natural environment. The observed limited growth at a salinity of 40 could be attributed to the organism's adaptation to the chemical composition of surface water in the coastal waters of Tanzania, where the average salinity is reported to be 35 (Mahongo and Shaghude, 2014; Painter *et al.*, 2021). Conversely, the observed underperformance and limited growth at

Table 3. The proximate composition of *Tetraselmis* sp. biomass harvested at different days. Mean ± SD, n = 6.

Composition (%)	Harvesting day		P-value
	Day 7	Day 16	
Crude protein	68.62 ± 2.97	63.97 ± 6.56	P = 0.143
Crude lipid	11.45 ± 1.4	9.09 ± 0.57	P = 0.003
Carbohydrate	6.49 ± 0.24	5.40 ± 1.16	P = 0.048
Ash	2.67 ± 0.18	8.63 ± 2.17	P = 0.001
Crude fibre	1.38 ± 0.05	1.20 ± 0.12	P = 0.016

Table 4. Quantity of minerals in *Tetraselmis* sp. biomass at different light orientation, and harvesting time. Mean ± SD

Mineral	Quantity of minerals (mg/100g)			
	Day 7	Day 16	Top-light	Side-light
Calcium	543.73±236.47	3847.25±784.12	2520.72±2248.09	1870.26±1382.35
Magnesium	349.98±123.01	403.06±52.43	408.73±58.65	344.30±116.79
Potassium	761.07±635.22	1223.34±10	1176.19±11	808.20±58
Phosphorus	1400.31±673.29	1245.89±129.42	956.71±187.36	1689.49±356.51
Iron	64.87±6.59	80.09±8.14	79.20±9.12	65.76±7.57
Selenium	0.002±0.000	0.013±0.019	0.003±0.002	0.012±0.02

salinity of 5 align with expectations, considering the marine nature of the species. On the other hand, the good performance of the organism at lower salinities of 15 and 25, compared to full strength, aligns with its natural environment. The estuary experiences periodic freshwater inflows, which correspondingly influences the organism capacity to thrive. The growth trend where no stationary phase was exhibited by *Tetraselmis* sp. indicates that the organism can be cultivated under laboratory conditions for an extended period. This unique characteristic opens avenues for diverse applications, enhancing its utility across various fields. Similar trend was reported by Hotos and Avramidou (2021) who also recorded an exponential growth without a stationary phase even at day 17 for the salinity of 20 and 40.

The findings regarding the effect of light on *Tetraselmis* sp. growth suggest that the organism was equally affected by light orientation. However, the highest cell density was achieved at higher light intensity (5.5±3.3 Klux). Light quantity and quality are important factors in regulating the productivity of photosynthetic organisms. Optimal growth is achieved when these organisms are exposed to light of optimal wavelengths (Schulze *et al.*, 2016). Lower light intensities below the saturation point have limiting effect on microalgae growth, which in turn, affects the synthesis of body composition (Hotos and Avramidou, 2021; Metsoviti *et al.*, 2019). The study conducted by Hotos and Avramidou (2021) reported a high growth rate of *Tetraselmis* sp., reaching up to $9.6x10^6$ cells/ml at a light intensity of 8.0 Klux. In contrast, at 2 Klux the maximum density attained was $2.2x10^6$ cells/ml (Hotos and Avramidou, 2021). A similar pattern was observed by Montes-González *et al.* (2021) in *Tetraselmis suecica,* where a high density of $26x10^6$ cells/ml was recorded under high light intensity compared to low intensity. Despite the similar trend in the findings, the cell density recorded in the current study is lower compared to previous research studies (Hotos and Avramidou, 2021; Montes-González *et al.*, 2021). The variation in the number of cells obtained might be due to differences in the strain used, and also other laboratory conditions including the type and quantity of nutrient, photoperiod, pH and salinity. When comparing the growth trend to other microalgae, such as *Chlorella vulgaris* (Metsoviti *et al.*, 2019), *Desmodesmus* sp. and *Scenedesmus obliquus* (Nzayisenga *et al.*, 2020), it was observed that growth increased with higher light intensity. The similarity in growth trends shows that *Tetraselmis* sp., like other photosynthetic microalgae, undergo rapid cell division at higher light intensities, resulting in higher cell count.

In terms of body compounds, it was found that low light intensity, coupled with early harvesting during the phase of steady growth, resulted in higher levels of protein, lipid, carbohydrate, and fibre content. This suggests that light plays a crucial role not only in growth but also in shaping the nutritional profile of *Tetraselmis* sp. biomass. This trend was also observed in other microalgae species, such as *Chlorella vulgaris* (Metsoviti *et al.*, 2019) where the quantities of crude protein and fibre remained constant as light intensity increased. However, in contrast to previous research (Nzayisenga *et al.*, 2020; Maltsev *et al.*, 2021), the findings regarding lipid production differed. Higher lipid content was recorded in *Chlorella* and *Scenedesmus* species under higher light intensities (Nzayisenga *et al.*, 2020; Maltsev *et al.*, 2021). In other species of *Tetraselmis*, previous research reported protein content within a range of 31 to 59 % (Schwenzfeier, 2013; Khatoon *et al.*, 2018; Wang *et al.*, 2021), which is lower than reported in this study. However, the values of carbohydrate (20 %) and lipid (22 %) reported by Schwenzfeier (2013) are much higher than those recorded in the current study. The content of lipid in the current study is also lower than the 28.3 % reported by Kim *et al.* (2016). The variation in the body composition of microalgae

depends on various factors, including the purpose of cultivation and the culture conditions. Previous studies have reported that microalgae tend to accumulate high levels of carbohydrate or lipids when the culture is deficient of nitrogen (Markou *et al*., 2012; Teo *et al.*, 2014; Kim et *al.*, 2016). In the current study, the microalgae were cultivated in a commercial medium (Guillard's F/2) without any manipulation of ingredients, explaining the variation in the contents of carbohydrates and lipids compared to previous studies.

Minerals are essentials for the normal processes and health of animal bodies though required in small quantities. In the current study, the biomass harvested on day 16 contained notably higher levels of minerals, particularly calcium, potassium, and magnesium. This observation indicates that longer cultivation periods may enhance the mineral content of *Tetraselmis* sp., potentially extending its nutritional value for aquaculture and other applications. In terms of total minerals (ash), the recorded amount is approximately half of the 15.2 % reported by Pereira *et al.* (2019) for *Tetraselmis* sp. produced at commercial scale. Despite the lower composition of ash content, the values fall within the comparable range (1.9 to 37 %) observed in most microalgae species (Liu, 2017). On the other hand, the high content of calcium, potassium, and magnesium in the biomass of *Tetraselmis* sp. harvested on day 16 implies the abundance of those minerals in the surrounding water. However, the variation of phosphorus levels in combination with other minerals can be explained in the context of the effectiveness of *Tetraselmis* sp. in removing phosphorus from the water (Patel *et al.*, 2012). The study by Patel *et al.* (2012) reported higher uptake rates of phosphorus from wastewater on day 8 compared to day 16, which is a similar time frame to the current study. The levels of trace minerals in the biomass of *Tetraselmis* sp. were low, as they are required in low quantity. Selenium plays an essential role as a strong antioxidant, protecting living cells against oxidative effects. In the human body, it slows aging, prevents cell damage, and boosts the immune system (Gojkovic *et al.*, 2015). However, the benefits of selenium are observed at lower doses, while high concentrations can generate reactive oxygen species that damage body cells (Sun *et al.*, 2014). For instance, Watanabe *et al.* (1997) recommended a range of 0.15 to 0.5 mg/kg of selenium per dry weight as suitable for proper fish growth. The presence of selenium in *Tetraselmis* sp. biomass in lower amouts is a good sign that the microalga is safe for consumers. Moreover, in aquaculture it indicates that when *Tetraselmis* sp. is used as a natural food, there is no need of fortifying the feeds with synthetic antioxidants.

Conclusions

The study revealed that *Tetraselmis* sp. exhibited optimal growth in moderate salinity levels of 15 and 35, highlighting its adaptability to local conditions within the Western Indian Ocean. Light intensity significantly influenced body composition, favouring higher protein, lipid, carbohydrate, and fibre content under low light and early harvest. Extended cultivation periods enhanced mineral accumulation, notably calcium, potassium, and magnesium. The study demonstrated that the local strain of *Tetraselmis* sp. can be kept for culture under laboratory conditions, and the establishment of optimal culture conditions can be set depending on the cultivation objectives. Therefore, this study concludes that, with a specific focus on *Tetraselmis* sp., there is significant potential for isolating and cultivating this local marine microalga for industrial applications.

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