Marine microalgae for outdoor biomass production—A laboratory study simulating seasonal light and temperature for the west coast of Sweden

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Abstract
At Nordic latitudes, year-round outdoor cultivation of microalgae is debatable due to seasonal variations in productivity. Shall the same species/strains be used throughout the year, or shall seasonal-adapted ones be used? To elucidate this, a laboratory study was performed where two out of 167 marine microalgal strains were selected for intended cultivation at the west coast of Sweden. The two local strains belong to Nannochloropsis granulata (Ng) and Skeletonema marinoi (Sm142). They were cultivated in photobioreactors and compared in conditions simulating variations in light and temperature of a year divided into three growth seasons (spring, summer and winter). The strains grew similarly well in summer (and also in spring), but Ng produced more biomass (0.225 vs. 0.066 g DW L−1 day−1) which was more energy rich (25.0 vs. 16.6 MJ kg−1 DW). In winter, Sm142 grew faster and produced more biomass (0.017 vs. 0.007 g DW L−1 day−1), having similar energy to the other seasons. The higher energy of the Ng biomass is attributed to a higher lipid content (40 vs. 16% in summer). The biomass of both strains was richest in proteins (65%) in spring. In all seasons, Sm142 was more effective in removing phosphorus from the cultivation medium (6.58 vs. 4.14 mg L−1 day−1 in summer), whereas Ng was more effective in removing nitrogen only in summer (55.0 vs. 30.8 mg L−1 day−1). Our results suggest that, depending on the purpose, either the same or different local species can be cultivated, and are relevant when designing outdoor studies.

1 INTRODUCTION

The demands for biomass as the feedstock for food, feed, oil-based materials such as polymers, and energy production are substantial and continue to rise worldwide, imposed by the increasing world population and the need for sustainable fossil fuel alternatives. Several resources of biomass have been considered, such as food crops, crop wastes, woody parts of plants, garbage, and microalgae. Microalgae have been used for human diet and wastewater treatment already since the 1950s, but there are many problems with productivity and harvesting to be solved in order to produce biofuels (Gilmour, 2019). They can be used either as an alternative or complementary sources of neutral lipids (triacylglycerols) in biofuels such as biodiesel, which mainly are produced from crop plants and industrial waste from paper
Microalgae are promising since they are at least fourfold more productive energy-wise than agricultural crops, such as rapeseed, have a shorter growth cycle, and require less surface, which does not have to be arable land (Ekendahl et al., 2018; Schenk et al., 2008). Moreover, microalgae cultivated in wastewater can recycle key nutrients (nitrate and phosphate) into biomass for animal feed, preventing eutrophication (Su, 2021).

When cultivated in outdoor facilities, microalgae experience similar type of environmental fluctuations to those in natural habitats, namely irradiance, temperature and nutrient availability. All may affect the biomass productivity and lipid content, as exemplified by an extensive simulation study of the global weather effects on a Nannochloropsis species (Moody et al., 2014), and confirmed by outdoor experiments (e.g., Matsumoto et al., 2017; Rodolfi et al., 2009). To ensure high productivity, it is necessary to select species and strains that are flexible, meaning, that they efficiently adjust their physiology (e.g., photosynthesis) to a changing environment. Exploiting the natural diversity of microalgae adapted to the local environment may be advantageous, particularly at Nordic latitudes (Cheregi et al., 2019). For example, the climate conditions on Sweden’s west coast vary between an average temperature of 0.5°C and irradiance of 200 μmol photons m⁻² s⁻¹ in January to an average of 19°C and 1500 μmol photons m⁻² s⁻¹ in July. Massive blooms of the diatom species Skeletonema marinoi (Sm) are observed annually on the west coast during February–March (Godhe & Härnström, 2010), implying that local microalgae can thrive even during the cold season. Different Sm strains succeed each other seasonally during the year in the Gullmar fjord (Saravanan & Godhe, 2010). In another study, a Sm strain from the Swedish west coast showed a higher thermal tolerance of growth than a strain from the Adriatic sea in the Mediterranean (Kremp et al., 2012).

A rotation of local microalgae species/strains could be the preferred strategy for biomass production throughout the year. However, despite their adaptation to certain seasons, are they able to efficiently produce biomass? Is the produced biomass rich in proteins, lipids and energy? It is important to understand that biomass alone is not a suitable parameter to compare different algae species or seasonal isolates. They will produce different amounts of biomass of different composition, resulting in widely differing energy contents. To address this problem, the total energy stored in the biomass can be estimated, specifically the total produced biomass per liter per day multiplied with the energy content of the biomass. Thus, the resulting energy productivity by the organisms makes it possible to compare microalgae species regardless of the growth season.

In this study, we aimed to find microalgae species/strains adapted to the seasons of the west coast of Sweden, that could be cultivated outdoors and produce biomass, intended as a feedstock for biodiesel, biomaterials, food and feed. We also aimed to verify the potential of selected microalgae strains for wastewater remediation, by evaluating the uptake rate of nitrate and phosphate from the cultivation media. To achieve these aims, from a collection of 166 Sm strains isolated during different seasons from various locations on the west coast of Sweden, we screened 51 strains for growth and the best 19 of them in terms of biomass yield and fatty acid content. One Sm strain was selected and compared with a local strain of Nannochloropsis granulata (Ng) in photobioreactors simulating the light and temperature on the west coast of Sweden during a year divided into three growth seasons.

## 2 Materials and Methods

### 2.1 Microalgae strains and cultivation for screening

The Sm strains used in this study (Table S1) were obtained from the culture collection GUMACC (Gothenburg University Marine Algal Culture Collection, https://www.gu.se/en/marina-vetenskaper/about-us/algal-bank-gumacc). The Sm strains were non-axenic, since the associated microbiome promotes growth of the host (Johansson et al., 2019). Ng, also non-axenic, was chosen without undergoing screening, as the only local strain in the GUMACC collection belonging to the Nannochloropsis genus.

Microalgae were maintained in the collection in aerated 30 ml flasks (polystyrene, Sarstedt), without shaking, at 10°C, an irradiance of 20 μmol photons m⁻² s⁻¹ and a 12-h photoperiod in a medium based on natural seawater collected from a 30-m depth at the Tjärnö Research Station at University of Gothenburg, Sweden. The seawater was filtered using a 0.7 μm GF/F glass fiber filter, the salinity adjusted with deionized water to 26, and sterilized by autoclaving at 121°C for 20 min. Nutrients of the standard f/2 marine cultivation medium (NaNO₃, NaH₂PO₄, microelements, vitamins, Guillard & Ryther, 1962) were added to the seawater. For the Sm strains, the seawater enriched with f/2 medium was further supplemented with 0.11 mM Na₂SiO₃.

To reactivate the strains kept in collection, working cultures of 20 ml (in 50 ml flasks) were prepared in a standard growth medium (filtered seawater autoclaved and enriched with f/2 plus Na₂SiO₃). The cultures were grown on a rotary shaker (60 rpm) at 16°C, an irradiance of 150 μmol photons m⁻² s⁻¹ and a 12-h photoperiod (hereafter referred to as standard conditions) for 6 days. The reactivation was repeated and represented the first screening allowing to select for growing strains. In the secondary screening, triplicate cultures of 20 ml were inoculated from the working cultures at a cell density of 0.5–1 relative fluorescence units (RFU) (~5–10 x 10⁴ cells ml⁻¹) and incubated in the same conditions as during reactivation, for 6–8 days. Algal growth was monitored by changes in chlorophyll fluorescence expressed in RFU using a Varioscan™ Flash Multimode Reader (Thermo Scientific) in multi-well microplates, as described by Gross et al. (2018). The fluorescence wavelength settings were 425 nm for excitation and 680 nm for emission detection, and the samples were dark adapted for 10 min at room temperature prior to the measurement. From the RFU data during the period of exponential growth, specific growth rates (μ) were calculated as follows:
ln RFUt2 − ln RFUt1
(t2 − t1)
where RFUt2 and RFUt1 are the relative chlorophyll fluorescence at two consecutive measurements between days 2 and 6 of the exponential growth. The conversion from RFU to cell number was done according to Gross et al. (2018). Strains that displayed at least 21 RFU (−2 × 10⁶ cells ml⁻¹) were selected for the third screening. The growth conditions were identical to those used in the secondary screening, but the culture volume was increased to 100 ml (in 500 ml flasks) for the purpose of generating enough cells for biomass and fatty acids determination.

2.2 | Microalgae cultivation in photobioreactors

Inocula of Sm142 and Ng strains were grown in 500 ml volume (in 1 L flasks) at room temperature using an irradiance of 150 μmol photons m⁻² s⁻¹ and a 12-h photoperiod. Each flask was aerated by bubbling with ambient air under magnetic stirring (120 rpm). The inoculum accounted for 5% of the final volume of the subsequent culture in 1 L photobioreactors (PBRs, double-walled wide necked borosilicate GLS80 glass bottles, outer diameter 110 mm, height 270 mm). The cultivation medium for PBR experiments was prepared as described for the laboratory screening; however, all components of the f/2 medium (NaNO₃, NaH₂PO₄, microelements, vitamins, and also Na₂SiO₃ in the case of Sm142), were added in 3-, respectively 14-fold excess through filter sterilization (0.22 μm, cellulose acetate filter, VWR).

According to records for air temperature, irradiance and sunshine duration for the west coast of Sweden during 2014–2016, we have designed three growth seasons for simulation: a longer winter (5 months: October–February), spring (3 months: March–May) and summer (4 months: June–September) (Figure S1). We have chosen to design three instead of four seasons taking into consideration the long-term goal of developing an outdoor cultivation system with minimal operation including rotation of algae species/strains. Nine PBRs, run in parallel, were illuminated using cold white LEDs controlled by a computer to simulate photoperiod and irradiance relevant for each seasonal condition. Irradiance was verified with a light meter (ULM-500 with US-SQS/L spherical sensor, Heinz Walz GmbH, Effeltrich, Germany). The double walls of the PBR allowed to maintain constant temperature of the cultures using a cooling water-bath and a refrigerator. For carbon supply, air enriched with 2% CO₂ was bubbled through capillaries into the bottom of each culture as needed to regulate pH. The pH was controlled separately for each culture by automatic injection with CO₂ when the pH increased above 8.0. To improve mixing of the cultures, magnetic stirrers were used.

Microalgal growth in PBRs was monitored by chlorophyll fluorescence measured every 2 days with a fluorometer (Fluoromax 4, Jobin-Yvon, Horiba Scientific) using a 3-ml quartz cuvette following adaptation of the samples to room temperature. The fluorescence wavelength settings were 425 nm for excitation and 680 nm for emission detection. Samples were dark adapted for 10 min prior to each measurement. Samples exhibiting fluorescence >10⁵ RFU were diluted with fresh culture media to ensure measurements in the linear range of the method. The starting culture concentrations was ~10⁵ RFU. Cultivation was stopped when highest and/or stable fluorescence levels were reached, and biomass was collected for analyses. The growth of Ng was also monitored using optical density (OD) at 750 nm following adaptation of the samples to room temperature. Cell viability was assessed based on microscopic observations and visual inspection of the cultures color.

2.3 | Biomass determination and analysis

The biomass yield was determined at the end of the growth curve when reaching highest and/or stable levels of chlorophyll fluorescence (corresponding in most cases to the stationary phase). In the laboratory (flask-scale) experiments, samples of 25 ml culture were collected on pre-combusted GF/F (47 mm) Whatman® filters; filters were washed with a double volume of 0.5 M ammonium formate and incubated at 100°C for 24 h, to allow the determination of dry weight (biomass) as described (Zhu et al., 1997). In the PBR experiments, we followed a similar procedure for dry biomass determination with the following modifications: 5–10 ml cultures were collected on pre-combusted GF/C (47 mm) Whatman® filters and washed with a double volume of 0.5 M ammonium carbonate. Filters were dried at 60°C for 24–48 h until constant weight, the dry weight was determined by gravimetry, and the yield was calculated as the dry weight in grams per liter. The daily productivity was calculated as the biomass yield at the end of the experiment divided by the duration (days). When a culture entered the stationary phase before the end of the experiment, the number of days between the start of the experiment and onset of the stationary phase was used as duration in calculations of the daily productivity.

The biomass collected on the last day of the PBR experiment was assessed for elemental composition. The samples were collected by centrifugation at 7000g for 15–30 min at the temperature of the experiment, stored at −20°C and later freeze-dried (Alpha 1–2 LDplus, Martin Christ) for 48 h. The elemental (C, H and N), ash and energy content analyses of the freeze-dried biomass were performed for 1 g grinded samples according to standard methods: SS-EN-ISO 16948 using an Elemental Analyzer (CHN 628, Leco), SS-EN-ISO 18122 using a thermogravimetric analyzer (TGA 701, Leco), and SS-EN-ISO 18125 using a Bomb calimeter (C5003, IKA). The proportions of proteins, lipids, and carbohydrates were based on a proximate calculation from the elemental analyses of ash-free biomass according to Gnaiger and Bitterlich (1984) using 5.78 as the protein conversion factor and 2% bound water. They developed a rather complex mathematical method for the analyses of biomass composition based on ash weight and CHN content of organic matter. In principle, it is based on removal of the protein part of the biomass using the protein conversion factor, then lipids and carbohydrates are calculated from the C:H ratio until all biomass has been divided into each class.
2.4 | Nutrient analysis

The amounts of nitrate (N) and phosphate (P) in the media were measured every 2 days during cultivation, with an ion chromatographic system composed of a conductivity detector (882 Compact IC plus with an 858 Professional Sample Processor, Metrohm AG) and an anion exchange chromatographic column (Metrosep Asup 5 858 Professional Sample Processor, Metrohm AG) and an anion exchange column. The amounts of nitrate (N) and phosphate (P) in the media were measured according to Nyman et al. (2009) on 15 min dark-adapted samples using a Phyto-PAM (Walz, Germany) by applying 14 increasing light intensities from 64 to 1480 μmol photons m⁻² s⁻¹, of 1 min each. The relative electron transport rate (relETR) was calculated as follows:

\[ \text{relETR} = \left( \frac{Fv}{Fm} \right) \times E \times 0.5 \]

where, \( Fv \) and \( Fm \) are the variable photosystem II fluorescence yield and maximal fluorescence yield, respectively, for cells at the end of each 1 min exposure, and \( E \) is the irradiance expressed in μmol photons m⁻² s⁻¹. A factor of 0.5 is applied to correct for the partitioning of electrons between photosystem I and photosystem II. ETR curves were fitted with the waiting-in-line function of Ritchie (2008) that allowed estimation of \( \alpha \) (maximal light use efficiency) and \( \text{relETR}_{\max} \) (maximal relative rate of electron transport). The light saturation index of photosynthesis (Lk) was calculated from \( \text{relETR}_{\max} \) divided by \( \alpha \).

2.6 | Light response curves of photosynthesis

In order to distinguish the photosynthetic capacity of the various strains, response curves of photosynthetic electron transport versus irradiance were measured according to Nyman et al. (2009) on 15 min dark-adapted samples using a Phyto-PAM (Walz, Germany) by applying 14 increasing light intensities from 64 to 1480 μmol photons m⁻² s⁻¹, of 1 min each. The relative electron transport rate (relETR) was calculated as follows:

\[ \text{relETR} = \left( \frac{Fv}{Fm} \right) \times E \times 0.5 \]

where, \( Fv \) and \( Fm \) are the variable photosystem II fluorescence yield and maximal fluorescence yield, respectively, for cells at the end of each 1 min exposure, and \( E \) is the irradiance expressed in μmol photons m⁻² s⁻¹. A factor of 0.5 is applied to correct for the partitioning of electrons between photosystem I and photosystem II. ETR curves were fitted with the waiting-in-line function of Ritchie (2008) that allowed estimation of \( \alpha \) (maximal light use efficiency) and \( \text{relETR}_{\max} \) (maximal relative rate of electron transport). The light saturation index of photosynthesis (Lk) was calculated from \( \text{relETR}_{\max} \) divided by \( \alpha \).

3 | RESULTS

3.1 | Screening in standard conditions

To identify the most productive among the 166 local Sm strains in the GUMACC collection, we performed experiments in flasks using sea-water+f/2 medium in standard light and temperature conditions. A number of 51 strains were viable under these conditions, and 19 of them reached at least 21 RFU (2 × 10⁶ cells ml⁻¹) at day 6 (Figure 1A.
Table S1). Moreover, they produced dry biomass in the range of 0.15–0.25 g L\(^{-1}\) (Figure 1B) and with a fatty acid content of 3–8% (Figure 1C). The Sm142 strain was the best performing in terms of biomass yield, fatty acid content, and also reproducibility in repeated cultivations (Table S1). This strain also displayed the best photosynthetic capacity, given the highest maximal relative electron transport rate and light saturation index for photosynthesis (\(\mu\text{ETR}_{\text{max}}\) of 232 \(\mu\text{mol e}^{-}\text{m}^{-2}\text{s}^{-1}\) and \(\text{Lk}\) of 877 \(\mu\text{mol photons m}^{-2}\text{s}^{-1}\), Table S1).

As compared to the chain-forming Sm species, having cells connected by an intricate silicate-based cell wall, \(Ng\) is an eustigmatophyte consisting of single small cells (2–4 \(\mu\text{m}\)) and simple ultrastructure. When cultivated in standard conditions, the \(Ng\) strain grew well, reaching a stationary phase at 6 days (Figure S2) and yielded significantly more biomass (0.6 g L\(^{-1}\)) which was significantly richer in fatty acids (35%) than all Sm strains (Figure 1B,C). The photosynthetic capacity of \(Ng\) was even higher than that of Sm142 (\(\mu\text{ETR}\) of 263 \(\mu\text{mol e}^{-}\text{m}^{-2}\text{s}^{-1}\) and \(\text{Lk}\) of 1080 \(\mu\text{mol photons m}^{-2}\text{s}^{-1}\), Table S1). When analyzing the fatty acid profile, we found that Sm142 had a significantly greater proportion of the long-chain polyunsaturated omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), whereas \(Ng\) was richer in monounsaturated species (Figure S3). Taken together, the data obtained in standard conditions allowed us to select the \(Ng\) and Sm142 strains for further experiments in PBRs simulating three growth seasons. A larger volume (1 L) in PBRs for algae cultivation also made possible to determine the chlorophyll content and elemental composition of the resulting biomass.

### 3.2 Performance in simulated summer

To investigate the seasonal effects on biomass production, we have designed light (irradiance and photoperiod) and temperature conditions for three growth seasons in a year on the west coast of Sweden: summer (June–September), winter (October–February), and spring (March–May) (Figure 2A based on Figure S1). First, the selected strains were grown in PBRs in simulated summer. To increase the biomass obtained in standard conditions (Figure 1B), we used seawater+f/2 medium enriched threefold with all components. The growth of Sm142 strain in terms of RFU reached highest level at day 7 (Figure 2B) and were collected at this time point. The fluorescence declined later on, as an indication of a rapid loss of viable cells. \(Ng\) continued to grow, reached the highest RFU at day 8, which remained stable until day 10, when the cultures were collected for biomass characterization. Despite the same specific growth rate (\(\mu\)), the biomass yield of \(Ng\) (~1 g L\(^{-1}\)) and daily productivity were significantly higher than those of Sm142 (two- to threefold, Table 1). Moreover, the measured chlorophyll values indicated a significantly energy-richer biomass for \(Ng\) (~28 MJ kg\(^{-1}\) DW). Taken together, when calculating the daily energy productivity of each species in the summer condition, \(Ng\) was close to fourfold more productive compared to Sm142 (Table 1). In parallel with growth, we have also monitored the concentration of nitrate (N) and phosphate (P) in the cultivation medium. The two strains displayed nonsignificant differences in the rates of removal from the threefold enriched medium (Table 1), which became fully depleted of both nutrients by days 6–7 of the experiment (Figure 2C).

Indeed, the biomass yield in the summer increased almost twice for both strains when enriching the cultivation medium threefold with nutrients (Table 1) as compared to standard conditions using non-enriched medium (Figure 1B). Next, we aim to obtain sufficient yields (at least 2 g L\(^{-1}\) for \(Ng\)) for biomass analyses. Based on the canonical Redfield 106C:16N:1P ratio for ocean phytoplankton (Redfield, 1934; Redfield, 1958), we determined an enrichment factor of 14 to supply the required amounts of N and P nutrients in the cultivation medium. In these conditions, the RFU of Sm142 rose and reached higher levels than in threefold enriched f/2 at day 7 (Figure 2B) before decline. The RFU of \(Ng\) continued to rise until day 14 and remained stable during the last 4 days of the experiment. The growth rates were not significantly different, but the biomass yield of \(Ng\) (~3 g L\(^{-1}\)) and daily productivity were significantly higher than those of Sm142 (three- to sixfold, Table 1). Taking also into account the significantly higher calorific value (~25 MJ kg\(^{-1}\) DW), the estimated daily energy productivity of \(Ng\) was fivefold higher than that of Sm142 (Table 1). In agreement with the lower biomass, Sm142 was significantly less efficient in removal of N than \(Ng\) (Table 1). Both strains removed only 25% of the available N from the medium at day 6, and only \(Ng\) completely removed it by day 14 (Figure 2C). Sm142 was significantly more efficient in the removal of P (Table 1) from the medium, which was completely depleted within 6 days as compared to 10 days by \(Ng\) (Figure 2C). In fact, P appears to be the nutrient whose complete removal overlaps in time with the loss of cell viability for Sm142, observation valid for both the threefold and 14-fold enriched media (Figure 2B,C). Since the biomass yield was significantly higher in the 14-fold enriched medium for both strains, we have used this enrichment factor also for comparisons in the winter and spring experiments.

### 3.3 Performance in simulated winter

The length of the simulated winter experiment (Figure 2A) was set to 23 days, when Sm142 growth reached as high RFU as during the summer (Figure 2B). In these conditions, Sm142 grew at a significantly higher rate than \(Ng\), yielding twice as much biomass (Table 1). The low light and temperature conditions impacted growth, as indicated by the significantly lower specific rates than in summer (Table 1). The biomass and energy productivities for Sm142 in winter were 20%–25% of the ones obtained in the summer, whereas for \(Ng\) they decreased below 5%. Nutrient consumption in winter was also largely reduced as compared to the summer treatment, and the rates were significantly higher in Sm142 as compared with \(Ng\) (at least twofold, Table 1). After 23 days of growth, the medium of Sm142 still contained ~40 and ~80% of the initial amounts of P and N, respectively (Figure 2C). In \(Ng\) cultures, ~70 and ~90% of P and N, respectively, remained in the media at the end of the experiment, consistent with the slower growth in winter of this strain.
3.4 | Performance in simulated spring

In simulated spring (Figure 2A), the duration of the experiment was set to 16 days, the time required by the Sm142 strain to reach about the same RFU as in the winter and summer conditions (Figure 2B). There was no significant difference in the specific growth rates of Sm142 and Ng, but the biomass yield and daily productivity of Ng were slightly and significantly higher (Table 1). Since the biomass of Ng was significantly richer in energy (~23 MJ kg\(^{-1}\) DW), the calculated daily energy productivity was twice the one of Sm142. An intermediate consumption of nutrients was observed for both strains in the spring as compared to the summer and winter treatments (Figure 2C). Like in the other seasons, Sm142 was more efficient in removing P than N, since at the end of the experiment, only 7% of the initial P remained in the medium as compared to the 20% in the Ng medium (Figure 2C), and this was also reflected in the significantly higher P removal rate (Table 1). N removal from the medium followed a similar trend in the two strains and at the end of the experiment, 70% of the initial N remained unconsumed.

3.5 | Biomass characteristics in simulated growth seasons

From elemental CHN analyses we modeled the composition of the dry biomass. The ash content significantly differed between strains and varied even among treatments in the case of Sm142 (range of 10–12% for Ng and 17–43% for Sm142, Table 1). Since the variability in the ash content impacts the proportions of the organic components (proteins, carbohydrates and lipids), we have chosen to compare the composition of the ash-free biomass between stains and treatments. In simulated summer when using threefold enriched nutrients, carbohydrates constituted almost 50% of the ash-free biomass of Sm142 (Figure 3, Table S2). The second largest component of the biomass
TABLE 1  Growth, biomass and nutrient removal during three simulated seasons

| Parameter                                      | Strain | Summer, ×3 | Summer, ×14 | Winter, ×14 | Spring, ×14 |
|------------------------------------------------|--------|------------|-------------|-------------|-------------|
| Specific growth rate (µ. day⁻¹)                | Sm142  | 0.50 ± 0.17 AA | 0.48 ± 0.17 AA | 0.18 ± 0.00 BA | 0.30 ± 0.00 AA |
|                                                | Ng     | 0.52 ± 0.02 AA | 0.46 ± 0.01 BA | 0.05 ± 0.00 BA | 0.33 ± 0.02 BA |
| Biomass yield (g DW L⁻¹)                       | Sm142  | 0.35 ± 0.02 AA | 0.46 ± 0.06 AA | 0.36 ± 0.02 AA | 0.49 ± 0.04 AA |
|                                                | Ng     | 0.99 ± 0.05 AB | 3.14 ± 0.60 AB | 0.16 ± 0.05 AB | 0.59 ± 0.03 AB |
| Biomass productivity (g DW L⁻¹ day⁻¹)           | Sm142  | 0.050 ± 0.002 AA | 0.066 ± 0.009 AB | 0.017 ± 0.001 AB | 0.004 ± 0.003 AB |
|                                                | Ng     | 0.124 ± 0.005 AB | 0.225 ± 0.043 AB | 0.007 ± 0.001 AB | 0.049 ± 0.002 AB |
| Calorific value (MJ kg⁻¹ DW)                    | Sm142  | 18.90 ± 0.00 AA | 16.60 ± 3.10 AA | 13.25 ± 1.06 A  | 14.55 ± 0.98 AA |
|                                                | Ng     | 28.43 ± 0.15 AB | 25.00 ± 0.00 AB | n.d.          | 23.46 ± 0.56 AB |
| Energy productivity (kJ L⁻¹ day⁻¹)              | Sm142  | 0.945 ± 0.002 AA | 1.095 ± 0.027 AA | 0.237 ± 0.001 AB | 0.596 ± 0.002 AB |
|                                                | Ng     | 3.525 ± 0.000 AB | 5.625 ± 0.043 AB | 0.164 ± 0.000 AB | 1.149 ± 0.001 AB |
| P removal rate (mg P L⁻¹ day⁻¹)                 | Sm142  | 1.60 ± 0.00 AA | 6.58 ± 0.32 AA | 1.07 ± 0.04 A  | 2.54 ± 0.18 AB |
|                                                | Ng     | 1.48 ± 0.10 AA | 4.14 ± 0.06 AB | 0.50 ± 0.00 B  | 1.98 ± 0.46 AA |
| N removal rate (mg N L⁻¹ day⁻¹)                 | Sm142  | 23.66 ± 0.05 AA | 30.80 ± 1.36 AA | 8.52 ± 1.11 BA | 16.92 ± 3.30 AA |
|                                                | Ng     | 24.71 ± 1.82 AB | 55.00 ± 1.00 AB | 3.35 ± 0.37 B  | 17.21 ± 3.44 AB |
| Ash content (% DW)                              | Sm142  | 17.00 ± 0.00 AA | 34.00 ± 10.00 BA | 43.00 ± 2.65 B  | 38.00 ± 1.70 BA |
|                                                | Ng     | 10.00 ± 0.20 AB | 10.00 ± 0.58 AB | n.d.          | 12.00 ± 1.00 AB |

Note: The selected Sm142 and Ng strains were cultivated in seawater supplemented with 3- or 14-fold f/2 in simulated seasons, as described in Figure 2. The specific growth rate (µ) was estimated during the exponential phase from the fluorescence data in Figure 2B. Biomass was collected for analyses at the end of the growth curve. The dry weight (DW) was measured and the biomass productivity was calculated as the DW yield divided by the duration (days) until onset of the stationary phase. The calorific value and ash content were determined as described in Methods, and energy productivity was calculated from biomass productivity multiplied by the calorific value. The calorific value and ash content of the Ng biomass in winter conditions could not be determined due to insufficient DW for analysis. *For the estimation of energy productivity for Ng in winter, the calorific value from spring was used. The nutrient removal rate was estimated from the curves in Figure 2C, as the difference between the initial and final concentration and divided by the duration of the experiment. Data are means ± SD from three to five biological replicates. Different lower-case letters denote significant differences among treatments for the same species (one-way ANOVA and Tukey’s post hoc test, P < 0.01; GraphPad Prism). Different upper-case letters denote significant differences between the two species for the same treatment (Student’s t-test with Welch’s correction, P < 0.01; GraphPad Prism).

was represented by proteins (34%). When using 14-fold enriched nutrients in summer, we found that the biomass contained more proteins (50%) at the expense of carbohydrates (34%), whereas the lipid content (16–17%) was not affected by the enrichment. In winter and spring conditions, we found a further and significant accumulation of proteins in Sm142 (up to 62%) of the ash-free biomass, without a significant change in lipids (11–12%).

For Ng, in simulated summer when cultivated in a threefold enriched medium, the biomass contained almost 60% lipids (Figure 3, Table S2). A 14-fold nutrient enrichment resulted in increased protein content (over 40%) and decrease in lipids (40%), whereas the lower content of carbohydrates was not significantly altered (18–19%). Because of the extremely low biomass yield (0.16 g L⁻¹), we could not determine the biochemical composition of Ng in simulated winter. In spring, it produced mainly proteins (65%), much less lipids, and also less carbohydrates than in the summer (24 and 11%, respectively, Figure 3). When comparing the two strains in summer and spring, Sm142 produced significantly more carbohydrates, Ng produced more lipids, and there was no significant difference in the protein content.

Elemental analyses of the dry biomass also allowed to calculate the C:N ratios (Table S2). In the samples collected from winter and spring in 14-fold enriched medium, a ratio C:N of ~5 was found for both strains. This value is close to the canonical Redfield ratio of 6.625 (Redfield, 1934; Redfield, 1958), and even closer to the ratio of 6 that was reported for microalgae living in cold, nutrient-rich and high-latitude regions (Martiny et al., 2013). In the summer, the C:N ratio increased to just above 8 for Sm142 in the threefold enriched medium. For Ng, the ratio increased to 8 in the 14-fold enriched medium, and to 16 in the threefold enriched medium. The better growth of the cells in the summer (Figure 2B) has resulted in higher N consumption in the media (Figure 2C and Table 1). N limitation appears more severe for Ng cells, likely due to different metabolic demands.

4 | DISCUSSION

Achieving high yields of biomass intended as a feedstock for biodiesel, biomaterials, food, and feed with minimum energy input throughout the year is an important factor for the success of microalgae, particularly to be able to compete with low-priced fossil oil (Gilmour, 2019). Outdoor cultivation can reduce the energy demands, but in order to ensure high productivity throughout the year, it is necessary to select local species and strains physiologically adapted to the seasonal changes. The cultivation conditions can be further manipulated by nutrient enrichment. An outdoor study performed in open pond PBRs
in Japan provided a successful example of stable year-round lipid production by rotating two oleaginous diatom species with different temperature demands: *Fistulifera solaris*, between April and October, and the cold-tolerant *Mayamaea* sp. JPCC CTDA0820, between November and March (Matsumoto et al., 2017). Notably, the temperature of the cold season was 10°C, which corresponds to the simulated spring conditions in our study. Based on a modeling study, Jorde et al. (2017) recommended rotation of cold-adapted species (cryophilic green algae) and warm-adapted species (mesophilic eustigmatophytes) for EPA production in North European countries, such as Germany and Norway. In our laboratory study, we examined how three combinations of light and temperature, mimicking one year on the west coast of Sweden, impact the performance of a local diatom strain (*Sm142*) and a local eustigmatophyte strain (*Ng*), in terms of growth, biomass and energy productivity, and nutrient removal from the medium. Below we compare the performance in culture of our strains to other studies.

Based on screening in flasks using seawater+f/2 medium in standard light and temperature conditions, we selected 19 *Sm* local strains with growth in terms of cells densities >2 × 10^6 cells ml^-1 at 6–7 days (Table S1). These cell densities are on average fivefold higher than those reported in the literature for *Skeletonema* sp. in the same medium, conditions and duration (Orefice et al., 2019; Wang et al., 2020). In fact, we observed a large intraspecific variation in growth among the 51 screened *Sm* strains (Table S1), and attribute it to different isolation sites, environmental conditions and genetic diversity (Godhe & Rynearson, 2017). Furthermore, the biomass growth of the 19 strains (0.15–0.25 g L^-1, 6–7 days, Figure 1B) was higher than that reported for a *Skeletonema* sp. strain from the NW Adriatic Sea in the same medium and conditions (0.045 g L^-1 at 8 days, 0.1–0.2 g L^-1 at 15 days, Bertozzini et al., 2013). In our GUMACC collection containing 166 *Sm* strains, *Sm142* was the one with the highest photosynthetic capacity, biomass yield and lipid content. This strain, isolated from Hakefjord (also named HakH), displayed above-average growth (measured as cell density) among 23 strains in a previous study (Gross et al., 2018). The physiological mechanism behind its outstanding performance remains to be investigated. Strains belonging to the high-yielding *Nannochloropsis* genus most often originate from habitats with different environmental conditions than where they are ultimately used for biotechnological purposes. Our local *Ng* strain was isolated from an eutrophicated fjord in the Skagerrak region of the Swedish west coast (Karlson et al., 1996). In our experiments, it produced more biomass (0.5–0.6 g L^-1, Figure 1B) than reported for 12 *Nannochloropsis* strains of worldwide origin.
cultivated in similar medium, conditions and duration (0.3–0.4 g L$^{-1}$, Slocombe et al., 2015). The cell and, particularly, chloroplast ultrastructure of Ng are distinct from those of species of the same genus, for example, Nannochloropsis oculata and Nannochloropsis salina (Karlson et al., 1996), and could explain its outstanding performance in biomass production.

Ng together with Sm142 were selected for further experiments in PBRs simulating three growth seasons at the west coast of Sweden. The maximal biomass productivity for both strains was obtained in summer, and could be attributed to the highest irradiance (average of 700 μmol photons m$^{-2}$ s$^{-1}$ for 15 h) and temperature (average of 18°C) among all seasons (Figure 2A). Climatic conditions affect the growth of phytoplankton throughout the year, and maximal algae growth is determined by the amount of solar radiation reaching their chloroplasts. Temperature and nutrients will only limit the potential growth. In our study, the daily irradiances applied to the cultures in PBRs were approx. 10 000, 5000 and 1000 μmol photons m$^{-2}$ day$^{-1}$ for summer, spring and winter, respectively. Assuming light as the only limiting factor for growth, the biomass productivity should have been reduced to 50% and 10% in spring and winter, respectively, relative to summer. The actual daily productivities of Ng were reduced much more than hypothesized, namely to 22% and below 5%, respectively. Since the N and P nutrients remained largely unconsumed at the end of the experiment (Figure 2C), the temperature most likely limited the growth of Ng in addition to the light. This is in agreement with the low growth rates reported during cold stress (5–15°C) for other Nannochloropsis species (e.g., Nannochloropsis salina, Gill et al. (2018)). The Ng strain used in this study belongs to a phytoplankton community dominating in spring and summer (Karlson et al., 1996). This may reflect a seasonal occurrence of the Nannochloropsis genus in the natural environment, as there are no studies reporting it in either winter or early spring.

Skeletonema can grow even at –2°C (Anderson & Rynearson, 2020) and produces blooms at 4–8°C during February–March, when the coastal waters are loaded with nutrients and the grazing pressure is very low (Allen et al., 1999). Indeed, although Sm142 performed less than Ng in summer, its daily productivities decreased to only 62% and 25% in spring and winter, respectively. This suggests that Sm142 is better adapted for growth at lower temperatures, and that Ng cultivation would require external heating of the outdoor facilities to boost its growth. In our simulations, we have kept constant the temperature of the seasons. If the growth facility would be placed on land, the temperature of the system will likely follow the temperature of the air. However, a more constant temperature can be achieved if the algae cultivation system is embedded in seawater, in the coastal regions. Our simulations are based on climate data for 2014–2016, and conditions, in particular the average temperature may rise in the future, which would be beneficial especially for growth of Ng. As for Sm142, it would be worth to investigate if an increase in irradiance in winter conditions would improve the growth and biomass production, particularly for high-value products such as the omega-3 fatty acids (Figure 53).

A significant increase (from 40 to ~60%) in the lipid content of the biomass was observed for Ng in summer when decreasing from 14 to 3 the nutrient enrichment factor (Figure 3). The lower C:N ratio of the biomass obtained in the threefold enriched medium (8.75 vs. ~16, Table S2) indicates nitrogen limitation of the cells (Liefer et al., 2019), that favored the accumulation of lipids. The threefold nutrient enrichment yielded lower Ng cell densities (Figure 2B), most likely resulting in excess light per cell unit, condition also known to stimulate lipid synthesis (Sforza et al., 2012; Simionato et al., 2011). It could also be argued that the extensive P limitation combined with the N limitation (Figure 2C) may have altered the biomass composition toward lipid accumulation. However, this possibility is excluded by a study using Nannochloropsis sp. (Mayers et al., 2014).

N limitation has also been reported to contribute to lipid accumulation in S. marinoi (Bortozzini et al., 2013). At the highest C:N ratio of 8.8 obtained for Sm142 in threefold enriched medium, the biomass contained significantly less lipids than Ng (17% in summer), implying differences in the genetics and physiology of the two species, that are worth exploring. In fact, the lipid content remained the same (16%) also for cells grown in 14-fold enriched medium, where N was not limiting but P was depleted already after 2 days. The fast disappearance of P could be due to the “luxury uptake” and accumulation of polyphosphate bodies in Sm cells (Solovchenko et al., 2019). Decreasing the N:P ratio of the growth medium from 100 to 10 in a Nordic strain of Skeletonema costatum eliminated P limitation and led to an increase in the N uptake (Myklestad, 1977). According to these studies, if the N:P ratio would be lowered below the Redfield value of 15 used in our study, the N uptake by Sm142 could become more efficient and stimulate accumulation of lipids. The manipulation of the N:P ratio in the cultivation medium for Sm142 and also for the Ng strain could be an effective strategy to change the composition of the biomass toward more lipids, to be used for, for example, in biodiesel production.

Any consideration of microalgae species for biodiesel feedstock has to take into account the physicochemical properties of the biomass: moisture, volatile matter, fixed carbon, ash content, and the calorific value. Cellular ash content, which represents on average 17% of the dry biomass, varies a lot between different classes of microalgae (Finkel et al., 2016). The two local strains selected in our study belong to distinct microalgal classes having different cell wall composition: Sm142 is a diatom and requires silica to build its nanopatterned shell, while Ng is an eustigmatophyte and fixes carbon into its cellulosic wall. Thus, diatoms have an ash content that has been approximated to 28–33% of dry biomass leading to underestimation of their cellular components. In our study, the Sm142 biomass contained about 20% ash in summer and twice as much in winter (Table 1). Our observations are in line with an increase in the silica cell quota at low growth irradiances (Noric et al., 2011), which normally occur during the winter season. Ng biomass contained only about 10% ash, in line with previous reports (Kent et al., 2015; Metsoviti et al., 2019).

Energy-efficient microalgae cultivation for biomass production can be achieved if solar light is efficiently used and the ambient temperature in different seasons is optimal for physiology and growth. Based on our laboratory study performed in controlled conditions simulating seasonal variations for light and temperature, the predicted
Predicted seasonal productivity. The seasonal biomass and energy productivities were estimated from experiments in 1 L photobioreactors, by multiplying the corresponding average daily productivities for seawater supplemented with 14-fold f/2 (Table 1) with the total number of days in each growth season (insets based on Figure S1). In this model, overall, most energy-rich biomass can be produced during summer using Sm142. Sm142 is a better biomass producer during winter but energy-wise is similar to Ng. In spring, the biomass and energy productivities of Ng are somewhat higher than for Sm142 but still lower in summer. Sm142 or Ng could be cultivated for different purposes throughout the year.

Energy productivity for Ng in the winter is 3% of the one in the summer, and the corresponding number for spring is 20% (Figure 4), so it would not be feasible to cultivate this strain outdoors throughout the year on the west coast of Sweden. The productivity predicted in winter for Sm142 is 1.4-fold of Ng, but is only 4% of Ng in the summer, and the corresponding number in spring is 10%, implying that Sm142 cannot be used for energy production throughout the year either, and a rotation of the two selected strains would neither be beneficial. Therefore, our laboratory data suggest that biomass for energy as biodiesel could be produced at industrial scale in outdoor systems on Sweden’s west coast only using Ng during summer. More research into producing biodiesel from microalgae on a large scale is required to address constraints such as stable year-round production as well as cell harvesting and oil extraction (Gilmour, 2019). Pilot outdoor studies, like the one using fresh water algae reported by Ekendahl et al. (2018), are required to validate our modeling data and also to test the energy efficiency of the cultivation system. Furthermore, there are advantages and disadvantages in using seawater versus fresh water for algae biomass production (Chew et al., 2018; Venteris et al., 2013), that need to be investigated in outdoor pilot studies from a techno-economic point of view.

The results from our laboratory-based study highlight the potential of local marine microalgae to produce biomass for various purposes. The answer to our question “Shall the same species/strains be used throughout the year or shall seasonal-adapted ones be used?” is that both strategies are worth pursuing. The Sm142 strain can be cultivated year-round to produce biomass as feedstock for biomaterials, food and feed. The strategy of seasonal rotation of species can be considered if Ng, richer in lipids, is cultivated during June–September for biodiesel production, whereas Sm142 is cultivated during October–May for biomaterials, food and feed. Both Ng and Sm142 can accumulate proteins and high-value fatty acids and at the same time can efficiently take up N and P, making them relevant for sustainable feed and food production. In addition, Sm142 has a silica shell, which is a new source of materials with UV-B protecting properties (Aguirre et al., 2018). Biomass production could be combined with recycling of valuable and world-limiting nutrients such as N and P from seafood farms and industrial wastewater with the help of our strains. Skeletonema marinoi thrives in nutrient-rich coastal waters throughout the world, and Nanochloropsis species are also widespread. Therefore, our model for the west coast of Sweden to cultivate different microalgae species/strains for different purposes in different seasons, could be modified by changing the strains or species to suit other coastal regions provided they have another local climate.

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AUTHOR CONTRIBUTIONS
Cornelia Spe tea, Anna Godhe, Otilia Chere gi, and Susanne Ekendahl conceived the project; Otilia Cheregi performed the screening, biomass determination, and analyzed the datasets. Mats X. Andersson performed the FAME analyses. Johan Engelbrektsson designed the simulated Nordic seasons, built the PBRs and performed the nutrient analyses. Niklas Strömberg performed the nutrient analyses and calculated the biomass and energy productivities. Otilia Cheregi, Niklas Strömberg, and Cornelia Spe tea wrote the manuscript draft. All authors commented and contributed to the final version of the manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon request.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

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