Enhancing hatchery production efficiency for commercial offshore cultivation of giant kelp (macrocystis pyrifera) in Luderitz, Namibia

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Short Report

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Abstract

*Macrocystis pyrifera* is a potential candidate for aquaculture because it grows rapidly, forms extensive underwater forests, and yields substantial biomass; and was the focus of this study. This study assessed sporophyte production by gametophytes of *M. pyrifera* obtained from three populations: California (CAL), South Africa (CAT), and the Falkland Islands (FL) in relation to environmental parameters. Factors examined included temperature (7.5, 10 - control, 12.5°C), irradiance (22.5, 30 - control, 37.5 µmol m⁻² s⁻¹), and gametophyte stocking densities (0.085, 0.114 - control, 0.142 mg cm⁻²). The data collected were the time required for sporophyte generation at weekly intervals and the quantities of sporophytes produced per cm². Results indicated that a temperature of 12.5°C accelerated sporophyte production across all populations, with outputs of 126.03 (± 101.44) in CAL, 694.44 (± 244.46) in CAT, and 1265.42 (± 448.30) in FL. At 7.5°C, no viable sporophyte production was observed for either CAL or CAT, whereas for FL, an increase in sporophyte quantities was observed (2142.49 (± 736.44)). In FL, reduced irradiance (22.5 µmol·m⁻²·s⁻¹) resulted in increased sporophytes outputs (1705.25 (± 657.28)), while the control irradiance (30 µmol·m⁻²·s⁻¹) was optimal for CAL (259.26 (± 203.16)) and CAT (1970.16 (± 888.01)). Gametophyte stocking density yielded the highest sporophyte outputs in CAT (1898.15 (± 534.67)) and FL (749.42 (± 359.09)) at the control treatment, while no differences were recorded for CAL between all density treatments. This study revealed that the optimization of sporophyte production through parameter manipulation enhances efficiency, and future research can explore increased lab temperature and its impact on sea recruitment rates.

1. Introduction

Kelps are large marine brown algae of the order Laminariales [1], with 36 different genera currently accepted [2]. Historically, the term “kelp” was used to describe seaweed that could be burned to obtain sodium carbonate (soda ash) [3]. These kelp species grow in dense populations and form a unique underwater forest habitat [4]. Several kelp species can grow to large sizes and exhibit high growth rates; for example, *Macrocystis* can grow at a rate of up to 60 cm a day, reaching up to 50 m or more [5], and *Nereocystis* can grow from depths of up to 30 m and form a canopy on the surface [6]. These kelp species are found along rocky coastlines in temperate waters between 5 and 20°C, with distribution limited by nutrient availability, light, and water motion [7]. Optimal growth occurs in cooler waters, with sufficient light for photosynthesis, high nutrient content (particularly nitrogen and phosphorus), and moderate water motion—excessive turbulence can uproot and/ or damage the kelp [8]. The characteristic brown color of kelp species is due to the pigment fucoxanthin, which boosts sunlight absorption efficiency, allowing kelp to live in deeper waters [9].

Kelp is versatile and valuable [7], and has been used for centuries for various purposes such as food [10], medicinal and pharmaceutical industries, as well as for agricultural products such as fertilizers [11]. Kelp is also used in the production of biofuels as well as in other chemical products, such as agar for laboratory usage [7]. In addition, kelp is used as a feed ingredient for farmed fish, helping to reduce dependence on wild fish stocks [12]. Today, the demand for kelp and its various products is growing;
therefore, kelp cultivation is becoming increasingly popular, offering new opportunities for coastal communities and contributing to the growth of the blue economy[13]. In the past, seaweed cultivation has been performed at sea, whereby spores were applied directly onto twine and deployed in water (14). This method proved to be inefficient as the carrier ropes would be populated with epifauna before the sporophytes developed, reduced, or prevented recruitment altogether [14].

Today, most seaweed farmers have employed the production of micro-sporophytes in land-based laboratories and then transported them to the sea, which has been proven to promote sporophyte survival during out-planting, enhancing kelp farm operations [14]. In addition, hatchery production by sporophytes ensures the production and supply of large quantities of seedlings [14]. This is because hatcheries operate under fully controlled environmental conditions such as temperature, irradiance, nutrients, photoperiod, salinity, and stocking density of biomes until young sporophytes develop [15]. Importantly, the propagation of kelp micro-stages is performed under sterile conditions to minimize contamination by epifauna and microalgae [15].

*Macrocystis pyrifera* (Linnaeus) C. Agardh (1820) is one of the top potential aquaculture candidates in the macroseaweed industry [16]. However, knowledge gaps in the optimization of hatchery production and at-sea cultivation of *Macrocystis* hinder commercial growth in the industry [17]. Therefore, this study aimed to identify the optimum temperature, irradiance, and gametophyte stocking density for the lab-phase cultivation of *Macrocystis* using gametophytes from three populations: California (CAL), Cape Town (CAT), and Falkland Islands (FL). The goal was to determine the best conditions for producing sporophytes from gametophytes in the hatchery, maximize lab outputs, and ensure the overall success of large-scale offshore farming operations in Namibia. The work presented here focused on the individual effects, rather than the interactive effects of each of the three parameters investigated (temperature, irradiance, and gametophyte stocking density). Although these induction parameters may act synergistically to influence induction success, each parameter has an independent influence on the success of sporophyte production from gametophytes [18].

Furthermore, *Macrocystis* is known to display a degree of morphological and phenological plasticity within the species, highlighting differences in responses to different environmental conditions [19]. However, no studies have been conducted to identify the requirements for culture at the hatchery level or to compare the growth response of geographically distinguished *M. pyrifera* populations grown under the same culture conditions after transfer to the ocean. Thus, it is not known whether *M. pyrifera* has ideal culture/induction requirements across populations. This study aimed to fill this knowledge gap to aid the success of Kelp Blue operations. This study might also complement Kelp Blues’ efforts to identify the dispersal and settlement risk of *Macrocystis* along the Namibian coastline. Additionally, the findings of this study may assist in restoration programs where naturally occurring *M. pyrifera* populations are in decline due to climate change and anthropogenic pressures.

2. Methodology
The present study was conducted at Kelp Blue’s land-based seaweed hatchery in Lüderitz. *M. pyrifera* gametophytes from three populations, California (CAL), South Africa (CAT), and Falkland Islands (FL), were sourced from Hortimare B.V., Netherlands, and were then transported to the Kelp Blue hatchery in Lüderitz, Namibia, and held under red light (1–5 µmol m\(^{-2}\) s\(^{-1}\)), at 10°C, with a single doze (1mL/L) of Varicon Aqua Cell-hi F/2P mix, and at high biomass to prevent them from undergoing unintended reproduction. Each variable was set at three levels: control, 25% increase from the control, and 25% decrease from the control. The study encompassed three replicates for each variable per population, totalling 81 experimental units. Each variable was examined independently, with the non-examined variables held constant throughout the experiment.

The experimental setup involved a containerized hatchery with adjustable cooling and metal shelves. Lighting units with shaded configurations were affixed to the shelves to control light exposure for gametophytes/sporophytes. All seawater used in the study was sterilized to minimize biological contamination. Collected from the ocean, it was treated with UV sterilization, mechanical filtration, and pasteurization using clean 5 L HDPE Jerry cans in a freshwater-filled 50 L hot-water urn. The urn was heated to 66–85 °C, and after maintaining ~ 70 °C for ~ 30 min, the water was rapidly cooled to ~ 10 °C for ~ 24 h, with two cycles. The induction trays and lids were sterilized by overnight soaking in a chlorine-based disinfectant. After thorough rinsing, labelling, and drying, the trays were placed under laminar flow, sprayed with 70% alcohol, dried for 5–10 min, and rinsed three times with sterile seawater to remove residual ethanol.

After induction, qualitative data on gametophyte reproduction were collected weekly for three weeks: “induction checks” on days 7, 14, and 21 (7D, 14D, & 21D). During induction checks, trays were inspected under a compound microscope, capturing two images per magnification (x40, x100, x400) for each replica. At the end of the three-week induction period, sporophyte quantification was performed by scraping them from trays, diluting samples, and counting individual sporophytes using a microscope, from which an inferal of the number of sporophytes produced per replica was deduced. Quantitative data were subjected to statistical analysis in R [20], involving the application of statistical tests such as Levene’s test and Shapiro-Wilk normality test. To identify significant differences, non-parametric tests, such as Kruskal-Wallis and parametric test ANOVA, were employed, followed by post hoc tests where necessary.

### 3. Results

#### 3.1. The effect of temperature on sporophyte production

Viable sporophyte production was observed at week two for the 12.5 °C treatment, and at three weeks in all populations for the control (10 °C), while no viable sporophyte production was observed at 7.5 °C treatment was observed for both CAL and CAT. Significant differences (CAL (p < 0.05), CAT (p < 0.05), and FL (p < 0.05), Kruskal-Wallis’s test) (Fig. 1) in the number of viable sporophytes produced among the different temperature treatments in all three populations were observed. For CAL, a Duncan's Multiple
Range post hoc test showed that there was no significant difference in the number of viable sporophytes produced at 10°C and 12.5°C ($p = 0.09$). For the CAT population, significantly more sporophytes were produced at 10°C than at 12.5°C ($p < 0.05$). In the FL population, significantly more sporophytes were produced at 7.5°C than at 10°C ($p < 0.05$) and 12.5°C ($p < 0.05$). The number of viable sporophytes in the FL population was significantly higher at 12.5°C than at 10°C ($p < 0.05$).

### 3.2. The effect of irradiance on sporophyte production

Irradiance analysis revealed no significant differences in the number of viable sporophytes produced among the three irradiance treatments in CAL ($p < 0.05$); however, significant differences were detected in CAT and FL ($p < 0.05$) (Kruskal–Wallis test). Duncan’s Multiple Range post hoc test revealed that in CAT, significantly more viable sporophytes were observed at 30 µmol m$^{-2}$ s$^{-1}$ than at 22. 5 µmol m$^{-2}$ s$^{-1}$ ($p < 0.05$) & 37.5 µmol m$^{-2}$ s$^{-1}$ ($p < 0.05$), but no significant difference in the number of viable sporophytes was detected between 22.5 & 37.5 µmol m$^{-2}$ s$^{-1}$ ($p < 0.05$). In FL, no significant difference in the number of viable sporophytes produced was observed between 37.5 µmol m$^{-2}$ s$^{-1}$ & 30 µmol m$^{-2}$ s$^{-1}$ ($p < 0.05$), however, significantly more viable sporophytes were observed at 22.5 µmol m$^{-2}$ s$^{-1}$ than 37.5 µmol m$^{-2}$ s$^{-1}$ ($p < 0.05$) and 30 µmol m$^{-2}$ s$^{-1}$ ($p < 0.05$) (Fig. 2).

### 3.3. The effect of irradiance on sporophyte production

The results on the effect of gametophyte stocking density on induction success, as revealed by the Kruskal–Wallis test, showed a significant difference between the stocking density treatments in CAL ($p < 0.05$). Significantly more viable sporophytes were observed at 0.142 mg cm$^{-2}$ than at 0.085 mg cm$^{-2}$ ($p < 0.05$), but there were no significant differences in the number of viable sporophytes produced at 0.114 mg cm$^{-2}$ (0.085 mg cm$^{-2}$, $p = 1.00$) and (0.142 mg cm$^{-2}$, $p = 0.05$) (Duncan’s Multiple Range post hoc test). ANOVA revealed a significant difference between the treatments in CAT ($p < 0.05$, $F = 12.0$) and FL ($p < 0.05$, $F = 1.04$). Tukey HSD post hoc test showed that in CAT, 0.085 mg cm$^{-2}$ had a significantly lower number of viable sporophytes ($p < 0.05$), but no difference in the number of viable sporophytes was observed at 0.114 mg cm$^{-2}$ and 0.142 mg cm$^{-2}$ ($p = 0.90$). In FL, 0.085 mg cm$^{-2}$ produced a significantly lower number of viable sporophytes ($p < 0.05$), while the number of viable sporophytes produced at 0.114 mg cm$^{-2}$ and 0.142 mg cm$^{-2}$ was not significantly different ($p = 0.51$) (Fig. 3).

### 4. Discussion

This project aimed to determine the optimal conditions for cultivating *M. pyrifera* gametophytes using California, Cape Town, and the Falkland Islands populations in the laboratory, with the goal of maximizing sporophyte production for large-scale offshore farming. The study revealed varying outcomes; all populations showed increased sporophyte production at higher temperatures, on the other hand, Cape Town and California experienced reduced production at lower temperatures, while the Falkland Islands had increased sporophyte production at 7.5°C. Significant differences were observed in
the induction success of gametophytes from these populations under varying irradiances \((22.5 \text{ } \mu\text{mol} \text{ } \text{m}^{-2} \text{ } \text{s}^{-1}, 30 \text{ } \mu\text{mol} \text{ } \text{m}^{-2} \text{ } \text{s}^{-1}, \text{ and } 37.5 \text{ } \mu\text{mol} \text{ } \text{m}^{-2} \text{ } \text{s}^{-1})\) and stocking densities \((0.085 \text{ } \text{mg} \text{ } \text{cm}^{-2}, 0.114 \text{ } \text{mg} \text{ } \text{cm}^{-2}, \text{ and } 0.142 \text{ } \text{mg} \text{ } \text{cm}^{-2})\), rendering all three hypotheses invalid. *M. pyrifera* gametophytes displayed a broad range of optimal induction temperatures. The optimal induction temperature for *M. pyrifera* gametophytes may be influenced by various factors, including the prevailing temperature of [21], gametophyte gametogenesis, and germplasm temperature [22].

The observed lack of induction success at 7.5°C for CAL and CAT over the three-week induction period and increased success for the FL population at 7.5°C compared to 10°C and 12°C could be attributed to the carry over effects of the cold water in which the FL population thrives: temperature range of 4°C to 9°C [23], whereas CAT and CAL are found within temperature range of 10°C to 15°C [24]. This suggests that for optimal sporophyte production, *M. pyrifera* gametophytes could be induced at temperatures within the average range of their source environment. This might explain why there was viable sporophyte production at 10°C and 12.5°C for CAL and CAT, as these temperatures fall within the average temperature ranges of these populations' locations, supports this. However, the lower limit of the average temperature range for a given strain can be used for gametophyte preservation, particularly for longer durations [25], instead of sporophyte production. This could explain why the 7.5°C was unsuitable for sporophyte production in CAL and CAT, which are adapted to higher temperatures compared to FL. Furthermore, lower temperatures have been found to enhance the health of male gametophytes in some instances [26], whereas higher temperatures can negatively impact male gametophyte health, especially for populations adapted to colder waters [27]. This may explain the higher sporophyte production at 7.5°C than at 10°C and 12.5°C observed in the FL population in this study. Good male and female gamete health is important because it ensures a balanced sex ratio for successful fertilization and the production of quality and healthy sporophytes [28]. This underscores the importance of colder temperatures in supporting male gametophyte health during gametogenesis and preservation [27]. However, in this experiment, an imbalanced sex ratio was observed across all populations, potentially leading to insufficient male structures to fertilize the eggs. The recommended sex ratio for seaweed propagation is 1:1 or 2:1 for female gametophytes [29]. In this study, the sex ratio was not determined.

In this study, gametophytes reared at 10°C showed that approximately 12.5°C was the optimal temperature for sporogenesis across all populations, leading to early reproduction with egg production starting in the first week and viable sporophyte production in the second week of the three-week induction period. Comparatively, other treatments initiated egg production in the second week and sporophyte production in the third week, indicating that induction temperatures could potentially be increased to the upper limit of the natural occurrence range to accelerate production timelines without compromising sporophyte output. An increase of approximately 2°C in gametogenesis/gametophyte preservation, as demonstrated in a study by [26], had a similar effect of accelerating sporophyte production, corroborating the findings of this study. Additionally, research by [22] showed that gametophytes maintained at 8°C produced sporophytes more rapidly at 6°C than those induced at 12°C, suggesting that lower gametogenesis temperatures may lead to lower optimal temperatures for...
sporogenesis. In another study on Laminaria digitata by [30], inducing gametogenesis at temperatures as low as 5°C resulted in sporophyte production within two weeks, further highlighting the importance of pre-treatment conditions for gametophytes. Overall, the FL population exhibited greater thermal plasticity in this study, with successful induction observed at temperatures ranging from 7.5°C to 12.5°C.

Understanding the effects of temperature on the early life stages of *M. pyrifera* is crucial for selecting suitable gametophyte strains for aquaculture [26]. The results of this study suggest that temperatures can be increased (within the strain's acceptable range or by ~ 2 °C above the gametogenesis temperature) to speed up production without affecting the output. For instance, the temperature for producing sporophytes can be raised from 10°C (control) to 12.5°C without reducing sporophyte numbers in all three populations. Studies by [31] and [24] also support these findings, with optimal sporophyte production occurring at approximately 12°C and 11–14°C, respectively. This explains why no reduction in sporophyte output was observed at 12.5°C compared to 10°C in the CAL population. The study's findings also highlight that early sporophyte production leads to quicker out-planting, as observed at 12.5°C, where holdfast formation began in the second week instead of the third week. This fast tracking of outplanting is beneficial for seaweed cultivation, ensuring timely and successive seeding, accelerating offshore farm expansion, and enabling a swift response to market demand. Higher temperatures can positively affect kelp microscopic development stages and recruitment post-out-planting in warmer cultivation environments. Although faster production is valuable, maintaining quality and quantity is crucial. Optimal temperatures should expedite sporophyte production and enhance output; however, close monitoring of induction progress is necessary for sporophyte health.

As sporophytes grow, they require more nutrients and space to meet their physiological requirements. In this study, sporophytes in the highest temperature treatment appeared pale, potentially because of nutrient depletion or reduced photosynthetic activity. Promptly moving sporophytes from enclosed trays once they reach a sufficient size is crucial to avoid fluctuations in water parameters that could exceed acceptable quality limits. While increased temperature accelerates sporophyte production, exceeding the induction threshold could reduce success; thus, it is important to monitor daily environmental conditions during the hatchery phase. Understanding the effects of temperature on *M. pyrifera*’s early life stages of *M. pyrifera* is key for strain selection and potential thermal priming in aquaculture [26].

Besides temperature, sporophytes also rely on light for growth, with optimal irradiance varying by strain, gametophyte source, and growth medium [32]. This study tested induction success at 22.5 µmol m$^{-2}$ s$^{-1}$, 30 µmol m$^{-2}$ s$^{-1}$, and 37.5 µmol m$^{-2}$ s$^{-1}$ for CAL, CAT, and FL populations. FL showed significantly more viable sporophytes at 22.5 µmol m$^{-2}$ s$^{-1}$, possibly because of adaptation to lower irradiance levels. The gametophyte mortality at 37.5 µmol m$^{-2}$ s$^{-1}$ across all populations may be due to a sudden increase in irradiance from to 1–5 µmol m$^{-2}$ s$^{-1}$ without acclimation. This resulted in reduced sporophyte outputs for CAT and FL compared to the control treatment (30 µmol m$^{-2}$ s$^{-1}$), which is likely linked to a decrease in reproductive cells. [33] found that light saturation varied with temperature; this
study only explored one temperature treatment (10°C) with higher irradiance, limiting insights into temperature-irradiance synergies.

Optimal stocking density is crucial for good yield, as both high and low stocking densities can limit production outputs. The indifference in the number of viable sporophytes produced at a high stocking density (0.142 mg cm$^{-2}$) in this study may be due to gametophyte mortality and egg mortality, leading to a reduction in reproductive cells and sporophytes. The observed mortality suggests potential overstocking, with a higher stocking density possibly leading to increased gametophyte presence at the end of the induction period, which could hinder fertilization. High stocking densities often reduce resource conversion efficiency, leading to growth and survival rate reductions, and water quality deterioration. Conversely, low gametophyte stocking densities can lead to dispersed cell distribution, potentially reducing the production efficiency. A spacing that is too large may limit the success of sperms reaching eggs during fertilization. The experiment revealed that a control stocking density of 0.114 mg cm$^{-2}$ (dry weight) was optimal for sporophyte production in *Macrocystis*.

This study aimed to optimize the production of *Macrocystis* hatchery sporophytes using gametophytes from different regions. Although that these populations belong to the same species, they exhibit variations in the induction outcome. Higher temperatures (12.5°C) accelerated sporophyte production in all three populations, whereas lower temperatures (7.5°C) were less successful for CAT and CAT. High stocking densities (0.142 mg cm$^{-2}$) increased mortality, whereas low stocking densities (0.085 mg cm$^{-2}$) lowered fertilization rates. Various irradiance levels (22.5–37.5 µmol m$^{-2}$ s$^{-1}$) were effective, with the control (30 µmol m$^{-2}$ s$^{-1}$) yielding the most sporophytes per population. Further research is required to understand the effects of elevated temperatures on sporophyte production. Optimizing sex ratios, spore extraction, and gametogenesis processes could also improve gametophyte quality. This study focused on basic environmental parameters, but additional factors such as nutrients, photoperiod, and irradiance quality are also essential. Overall, enhancing hatchery kelp production is crucial for the aquaculture sector, ensuring a reliable supply of high-quality kelp seedlings while reducing the stress on natural kelp ecosystems. Hatchery methods support genetic diversity preservation and selective breeding to adapt to changing environmental conditions, restore depleted wild kelp populations, and promote biodiversity and economic growth.

**Declarations**

**Acknowledgement**

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**Competing interest** This manuscript is a subsection of HA Andreas’s Master’s thesis at the University of Namibia. The work presented here is our original work and has not been published previously. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with regards to intellectual property. We followed the regulations of our institutions regarding intellectual property.

**Data availability** Data is available on request

**Code availability** program R (R Core Team 2023)

**Author contributions** All authors contributed to the study’s conception and design.

Hilinganye A. Andreas: Conceptualization, Methodology, Investigation, Formal Analysis, Writing - Original Draft.

Michael J. Fleischman: Conceptualization, Methodology, Writing - Review & Editing.

Iitembu A. Johannes: Review & Editing, Supervision.

**Ethical approval** All authors read and approved the final manuscript.

**References**


Figures
Figure 1

The number of sporophytes produced per cm$^2$ from the three temperature treatments; 7.5 °C, 10 °C and 12.5 °C for three populations; California - CAL, Cape town – CAT and Falkland Islands – FL, quantified at the end of the 3-week induction period. Significant results are denoted by letters above each bar.

Figure 2

The number of sporophytes produced per cm$^2$ from the different light regimes; 22.5 µmol m$^{-2}$ s$^{-1}$, 30 µmol m$^{-2}$ s$^{-1}$ and 37.5 µmol m$^{-2}$ s$^{-1}$ for the three populations; California - CAL, Cape Town – CAT and
Falkland Islands – FL, quantified at the end of the 3-week induction period. Significant results are denoted by letters above each bar.

![Bar chart showing the number of sporophytes per cm² produced from the gametophyte stocking density treatments; 0.085 mg cm⁻², 0.114 mg cm⁻² and 0.142 mg cm⁻² for all three populations (California - CAL, Cape Town – CAT and Falkland Islands - FL), quantified at the end of the 3-week induction period. Significant results are denoted by letters above each bar.](image)

**Figure 3**

The number sporophytes per cm² produced from the gametophyte stocking density treatments; 0.085 mg cm⁻², 0.114 mg cm⁻² and 0.142 mg cm⁻² for all three populations (California - CAL, Cape Town – CAT and Falkland Islands - FL), quantified at the end of the 3-week induction period. Significant results are denoted by letters above each bar.