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CULTURE IN A CONTROLLED ENVIRONMENT OF SPOROPHYTES OF *Macrocystis pyrifera* IN THE PROVINCE OF ILO – MOQUEGUA - PERU

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Abstract: The use of macroalgae has increased in recent decades in human activities, *Macrocystis pyrifera* is one of the most important algae resources in terms of biomass on the planet. The species that support the brown macroalgae fishery in Peru are: *Macrocystis pyrifera* and the algae of the genus:*Lessonia* (*L. trabeculata y L. nigrescens*) forming large extensions of subtidal and intertidal meadows. The aim of this study is to describe and evaluate the development of gametophytes and sporophytes of *M. pyrifera*, under laboratory conditions. Fertile sporophylls from natural populations in the Ilo province, Compañía Amphibia area, were subjected to cleaning, water stress, and induction of sporulation, obtaining a homogeneous solution of zoospores, which were inoculated and spores sown. The experiment was carried out in a controlled environment of the Las Brisas Association, where two tests were carried out with a temperature of 16 ± 2 °C, a photoperiod of 12:12 and a light intensity of 2300 lux. Filtered seawater was used as a culture medium. enriched with Provasoli Medium, (30 ml lt-1) renewed weekly. Spore density and sporophyte growth rates were determined using an integrated microscopy system. Densities were 86,000 spores for the first trial, 76,000 for the second trial, performed in a Neubauer chamber and observed in a compound microscope with a 10x eyepiece. The gametophytic phase (microscopic) developed during 4 days of culture. From day 14, the development of the first sporophytes begins, which presented an average growth rate of 8,73 % day^{-1} and the average length reached at the end of the first trial (62 days of culture) was 3407 μm, with r2 = 0.91 and for the second trial the average growth rate was 8.85 %.day^{-1} and a length of 1352 μm with r2 = 0,98.
Keywords: *Macrocystis pyrifera*, cultivation, ilo.

INTRODUCTION

The use of macroalgae has increased in recent decades in human activities, as a nutritional supplement, food for other cultivated marine species, and obtaining phycoloides has allowed Chile to increase its income from this item in recent years. 7.5 to 57.6 million dollars (Ávila et al. 2001)

Macrocystis pyrifera is one of the most important algae resources in terms of biomass on the planet. The genus presents a bipolar geographic distribution (North 1971), commonly called: Giant Kelp (USA), Giant Kelp (Mexico), String kelp (Australia), Huiro (Chile) and Sargasso or bolita (Peru), it has a brown coloration, brown and can measure up to 30 meters in length (Schiel and Foster 2015).

The species that support the brown macroalgae fishery in Peru are: *Macrocystis pyrifera* and the algae of the genus: Lessonia (*L. trabeculata y L. nigrescens*) forming large extensions of subtidal and intertidal meadows (IMARPE 2012). These species of macroalgae are of ecological importance in the environment where they inhabit, and are considered bioengineering species, because they allow them to house and protect biodiversity of organisms, including fish, algae, molluscs, among others (Adami and Gordillo 1999; Palacios and Mansilla 2003, Plana et al. 2007 and Vásquez et al. 2012). According to fishing statistics on commercial marine macroalgae in Peru, between the period 2009 - 2017, *Macrocystis pyrifera* represented 86% (136690.9 t) of the total volume of macroalgae harvested.

The “kelps” algae industry moves figures of around 4 million tons of biomass, destined for the food and pharmaceutical industry, whose raw material comes from cultivated meadows, mainly from countries such as China, Japan and Korea or from natural populations present in Europe, North or South America (Kloareg

et al. 1999).

Considering the high content of essential amino acids and fatty acids, the protein and lipid quality of kelp flour such as sargassum is comparable to other vegetable sources (Cruz et al. 2000), which undoubtedly justifies the study. and the optimization of technologies for the development of the cultivation of *M. pyrifera* in our country.

Finally, it is important to solve the knowledge of the reproductive process of *M. pyrifera* under laboratory conditions, which will guarantee future successful cultures, knowledge of both the microscopic stages and their first stages of development is essential. Therefore, we consider that the development of knowledge with methods of the first reproductive phases of *M. pyrifera* is an important contribution to the cultivation process of this resource of great commercial importance.

The present study aims to establish the methodologies to develop the first phases of growth and the stages of development of the life cycle of *M. pyrifera* from the province of Ilo, Moquegua - Peru, under laboratory conditions.

MATERIALS AND METHODS

Fertile sporophylls of *M. pyrifera* were collected by freediving at an average depth of 2 meters in the natural populations of the Amphibian Company of the Ilo province. The adapted methodology was from the IMARPE brown algae cultivation manual (2019).

The cultures were evaluated by using a composite microscopy system and from obtaining the zoospores to the first sporophytes. Through this system we proceeded until the settlement, duration of the gametophytic phase (microscopic) and sporophytic phase of: *M. pyrifera*. In the same way, the length was determined each week from the settlement of the sporophytes, to determine the growth rate using the expression indicated by Hansen (1980):

Specific Growth Rate (% day^{-1}) = $100 [Ln(N_t/N_o)]/t$

In which:

N_o = initial size

N_t = final size

t = time interval in days

COLLECTION

Fronds are collected from the subtidal zone through freediving; for this purpose, it Fronds are cut with a knife and stored in an anchovetera mesh bag or "capacho"; they are moved to the shore, where those adult sporophytes with the presence of sori (reproductive structure) are selected (IMARPE 2019).

CLEANING AND TRANSFER

The sections of the fronds with the presence of sporangial sori were cut, submerged in drinking water and carefully brushed with a soft bristle brush in order to remove any epiphytic organism, avoiding damaging the tissue; distributed on absorbent paper stretchers and arranged inside an isothermal box in dark conditions and with cooling gel to maintain the temperature similar to their habitat; it is transferred to the laboratory in the shortest possible time (IMARPE 2019).

WATER STRESS

In the laboratory the temperature is recorded, the refrigerant gel is replaced, and the material biological is maintained under water stress at 15°C and darkness for 18 hours (IMARPE 2019).

INDUCTION TO SPORULATION

Through rehydration of reproductive tissue after 18 hours of exposure to dehydration, they were induced to sporulate zoospores in plastic containers with 2L of sterile seawater and fertilized with Provasoli, where 100

grams of biological material at 15°C under dark conditions and without air for 24 hours (IMARPE 2019).

SPORE COUNT AND RELEASE

It is possible to observe the release of the first spores after 2 hours of rehydration of the sori, so counts are preferably carried out at the time of rehydration, at 2 and 24 hours post induction; Samples are taken with a sterile pipette that are placed in the Neubauer chamber for observation with a 10x compound microscope; during this period, they are kept in dark conditions at 15°C and without air (IMARPE 2019).

INOCULATION AND SEEDING OF SPORES

The spore broth previously filtered through a sterile 75µ sieve for the passage of epiphytic organisms that affect the crop; it is inoculated and planted on artificial substrates that favor the settlement of zoospores when they lose their flagella and develop the germination tube (after 24 hours of rehydration) (IMARPE 2019).

GROWING CONDITIONS

In the initial phase, settled spores are kept at 15°C, with a 12:12-hour photoperiod. light/dark, 2500 to 2800 lux, constant aeration with moderate flow, enriched with 30ml^{-1}L of Provasoli (Starr and Zeikus 1993) and refills of 100% of the water that is replaced weekly (IMARPE 2019).

RESULTS

Development of spores and obtaining gametophytes.

The tests carried out in the first semester of 2022 to obtain the spores, showed a good state of maturity of the collected biological material, where zoospores were obtained and the culture conditions are shown in Table 1.

Temperature	16 ± 2 ° C
Illumination	2300 lux
Photoperiod	12:12
Culture medium (Provasoli)	30ml^{-1}L

Table 1: Initial culture conditions in a controlled environment

For the first trial there was a density of 86,000 spores, for the second trial it was 76,000, likewise, for which the spore counts were performed at the time of rehydration, and at 2 and 24 hours post induction, this is done in a Neubauer chamber and its observation in a compound microscope with a 10x eyepiece (Fig. 1).

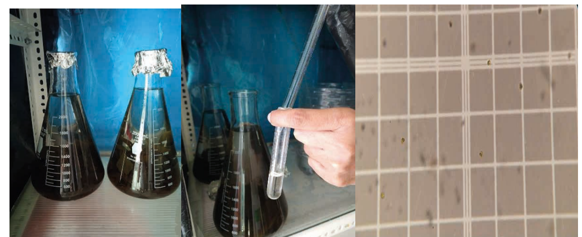

Fig. 1: Sampling of spores and counting in a Neubauer chamber

Germination and spore attachment

From 15 hours post inoculation, it is possible to demonstrate the presence of the peduncle in the spores, which lose motility and precipitate to settle in the circular collectors arranged vertically, where they remain for around 8 days with water exchange every 2 days. with moderate air, photoperiod 12:12 (light: dark), 2300 lux of light (blue light), 17°C and Provasoli nutrient medium.

The sowing of spores is carried out in polypropylene ropes 5 m long and 12 mm thick, wrapped in polyvinyl chloride (PVC) tubes 2” in diameter by 20 cm in length, arranged vertically in containers with 5L of drinking water. barren sea (Fig.2).

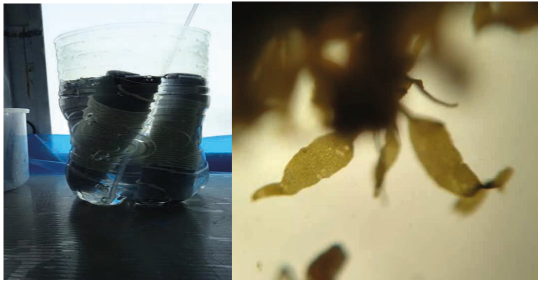

Fig. 2 Attachment and germination of spores

Development of male and female gametophyte phase

During the second week of cultivation, water exchange is avoided until day 12-15, the period in which the gametophytes fertilize (it will depend on the fertility of the gametophyte) and the female (fig. 3) and male (fig. 4) gametophytes are distinguished.

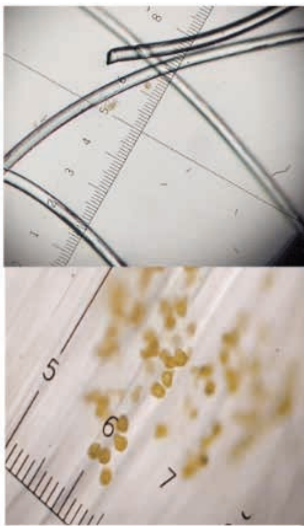

Fig.3: female gametophytes

Fig.4: male gametophytes

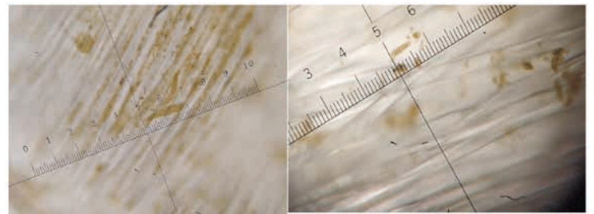

Fig.5: Fertilization

Between days 14 and 16 the beginning of the sporophytic phase of *M. pyrifera*, with the first divisions of the fertilized oogonium (Fig. 5), later a second division can be seen perpendicular to the first from which the development of the sporophytic phase begins (Fig. 6), where densities of 79561 and 70200 were obtained. for the first and second trials respectively.

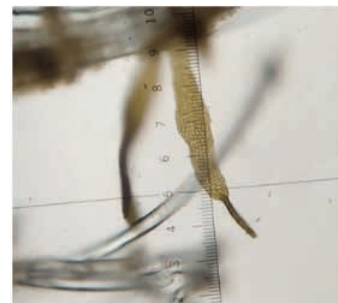

Fig.6: Sporophyte of *Macrocystis*

In this period the increase in length of the stems occurred exponentially and = $62{,}438e^{0.6248x}$; r2= 0,91. The average daily

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growth rate in frond length was 8.73 % day-1 (table 2) (Fig. 7-8) until completing an average length of 3407.89 μm, obtaining a maximum of 5000 μm and a minimum of 2000 μm for the first test.

Crop days	14	28	36	42	50	56	62
Average	47.19	236.77	972.41	1312.90	1586.67	1901.67	3407.89
Standard deviation	12.50	208.72	125.77	277.78	311.82	200.64	820.80
Standard mistake	2.21	37.49	23.36	49.89	56.93	36.63	129.78
N	32	31	29	31	30	30	40
Minimum size	30	90	700	600	1000	1500	2000
Maximum size	70	710	1200	1800	2100	2300	5000
TCD (% día^{-1})	11.52	17.66	5.00	2.37	2.59	9.72	*8.73

Table 2. Mean length (μm) of sporophytes and TCA daily growth rate (% day-1). (*) average growth rate during the entire cultivation period. First essay.

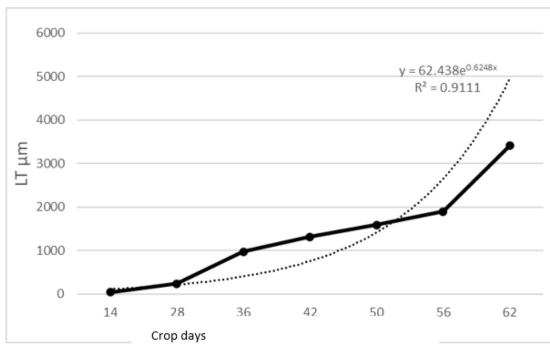


Fig.7: Incremento Longitud promedio μm

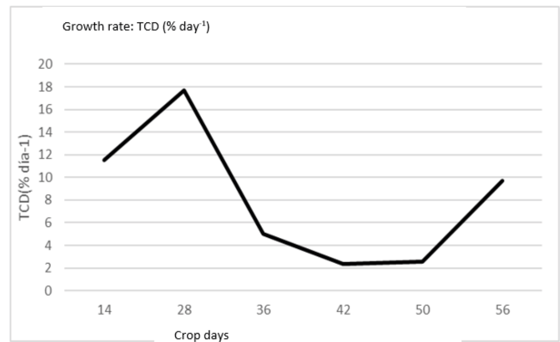


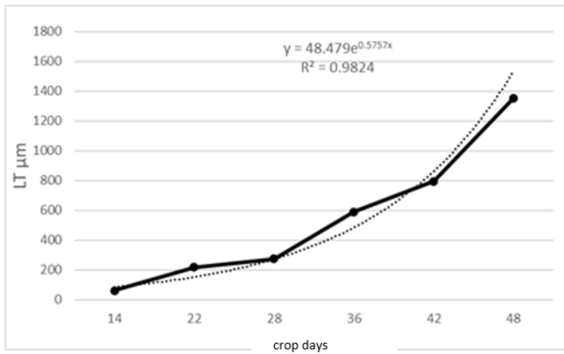
Fig.8: Tasa de crecimiento(%dia-1)

For the second trial, the growth of the stems was also exponential and = 48,479e$^{0.5757x}$; r2= 0,98. The average daily growth rate in frond length was 8.85 % day-1 (table 3) (Fig. 9-10) until completing an average length of 1352.08 μm, obtaining a maximum of 1600 μm and a minimum of 1000 μm (table 3).

Crop days	14	22	28	36	42	48
Average	61.05	217.50	273.89	590.00	796.30	1352.08
Standard deviation	7.92	42.32	32.40	91.53	140.69	150.71
Standard mistake	1.40	7.48	5.73	17.00	27.08	30.76
N	32	32	32	29	27	24
Minimum size	40	100	180	400	550	1000
Maximum size	90	420	360	760	1200	1600
TCD (% día^{-1})	15.88	3.84	9.59	4.28	8.82	*8.85

Second essay.

Table 3: Mean length (μm) of sporophytes and TCA daily growth rate (% day-1). (*) average growth rate during the entire cultivation period.



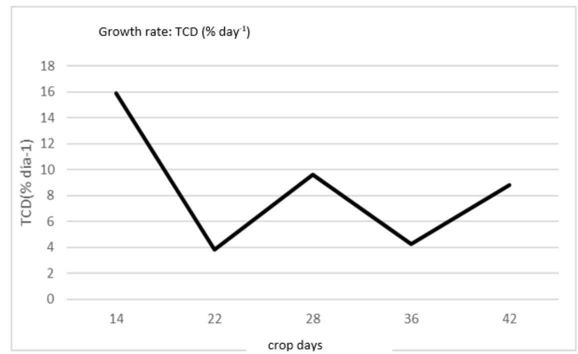


Fig.9: Average Length Increment: µm

Fig.10: Growth rate(%day-1)

DISCUSSION AND CONCLUSIONS

The sporophylls of *Macrosistys pyrifera* collected in autumn, they presented sufficient amounts of mature sori with abundant zoospores to obtain spores. This information is similar to the studies by Barrientos & Alveal (2001), they affirm that the spore processes for the Beagle Channel-Cape Horn develop only during the autumn and winter periods.

However, the studies by Candia et al. (1979), Avila et al. (1982) and Alveal et al. (1995) and Palacios & Mansilla (2003) the mature sori were in the spring period.

The density of zoospores, obtained in the spore release process, was 86,000 and 76,000 zoospores ml-1 for the first and second trials, respectively. Few authors document this information, but Palacios & Mansilla (2003) report 120,000 zoospores ml. -1 in the spring season, which would be carrying out studies for that season.

Regarding the period of settlement of zoospores, observed after 3:00 p.m., the structure of the settlement of the peduncle in the spores can be observed, allowing their motility and precipitation to be fixed in the collectors; in observations made by Celis (2003) who describes the formation of a lateral filament two days after the start of their cultures for settlement, information that is in contrast to the report by Palacios & Mansilla (2003) regarding the period of zoospore settlement, observed on the fourth day and the formation of the settlement structure that occurs between the 9th and 11th days of culture, when the filaments begin to differentiate gradually, and it is concluded that this phase lasts between 5 and 7 days.

Regarding the success of the culture in a controlled environment, a good mobility and viability of zoospores was observed, manifested in the large number of gametophytes and subsequent sporophytes obtained. From day 12 of culture, clear differences between male and female gametophytes are observed, a phase that is completed in a period of four days. Alveal et al. (1982) in studies of the life cycle of *M. pyrifera* comment that only after 20 days of culture it is possible to differentiate between male and female gametophytes; for which an early differentiation of the gametophytes would occur for the port of Ilo.

At the end of the cultivation trials in a controlled environment (76 days of cultivation), fronds of 5000 µm were obtained, sizes much higher than those obtained by Candia et al. (1979) and Celis (2003), where sizes of 400 µm and 2750 µm were not exceeded for 240 and 195 days respectively; what was reported by Palacios & Mansilla (2003) was 4242.03 µm in 65 days, which is

practically similar to the present study.

The high growth rates presented undoubtedly denote genetic and biological characteristics of *M. pyrifera* for the province of Ilo, positioning it as an important resource to be cultivated and with a different added value in the market.

RECOMMENDATIONS

Finally, it is suggested to carry out experiments that allow simultaneously comparing plants of *M. pyrifera* of Ilo with those of Ica and Arequipa with respect to reproductive potential (density of zoospores), duration of the phases of mobility, settlement and production of sporophytes, determining if among said populations any of them present comparative advantages for their use in successful cultures.

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