plants

MDPI

Article

Vegetative Propagation of the Commercial Red Seaweed *Chondracanthus chamissoi* in Peru by Secondary Attachment Disc during Indoor Cultivation

Samuel Arbaiza, Jose Avila-Peltroche, Max Castañeda-Franco, Arturo Mires-Reyes, Orlando Advíncula and Paul Baltazar *

Laboratorio de Investigación en Cultivos Marinos (LICMA), Dirección General de Investigación, Desarrollo e Innovación, Universidad Científica del Sur, Lima 15067, Peru
* Correspondence: pbaltazar@cientifica.edu.pe

Abstract: *Chondracanthus chamissoi* is an edible red seaweed with a high hydrocolloid content and food industry demand. This situation has led to a decline in their populations, especially in Peru. An alternative culture method based on the formation of secondary attachment discs (SADs) has shown several advantages over traditional spore strategies. However, there are still scarce reports of the SAD method in Peru. This work aimed to evaluate the best conditions for *C. chamissoi* maintenance prior to SAD development and the effect of locality on SAD formation using scallop shells as a substratum. Experiments were conducted with material collected from five localities in Pisco (Ica, Peru). Our results showed that the best conditions for *C. chamissoi* maintenance were: (1) fertilized seawater with Bayfolan® (0.2 mL L^{-1}); and (2) medium exchange every two days or weekly. These conditions reduced the biomass loss to 9.36–11.14%. Most localities showed a similar capacity to produce SADs (7–17 SADs $shell^{-1}$). However, vegetative algae, especially Mendieta, tended to present a higher number of SADs. Vegetative fronds also showed lower levels of necrosis and deterioration compared to cystocarpic and tetrasporophytic samples. This study shows the technical feasibility of culturing *C. chamissoi* through SADs for developing repopulation and/or intensive cultivation projects in Peru.

Keywords: carrageenophyte; locality; maintenance; Peru; seaweed cultivation; vegetative propagation

check for updates

Citation: Arbaiza, S.; Avila-Peltroche, J.; Castañeda-Franco, M.; Mires-Reyes, A.; Advíncula, O.; Baltazar, P. Vegetative Propagation of the Commercial Red Seaweed *Chondracanthus chamissoi* in Peru by Secondary Attachment Disc during Indoor Cultivation. *Plants* **2023**, *12*, 1940. https://doi.org/10.3390/plants12101940

Academic Editors: Loretto Contreras-Porcia and Manoj Kumar

Received: 18 February 2023
Revised: 20 April 2023
Accepted: 20 April 2023
Published: 10 May 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The edible red seaweed *Chondracanthus chamissoi* (C. Agardh) Kützing, traditionally known as "yuyo", "mococho" or "chicoria de mar", is distributed along the Pacific coast of South America (5–42° S) and considered endemic to the coasts of Peru and Chile [1], although there are some reports in distant regions, such as Korea, Japan, and France [2,3]. This species grows in the low-intertidal to subtidal zones (15 m depth), attached to rocky and calcareous substrates by a basal disc [4,5]. Due to its morphological variety, three forms were formerly described: f. *chauvini*, f. *lessoni*, and f. *glomeratus*. However, all of them are currently synonymized with *C. chamissoi* [3,6,7].

In Peru, *C. chamissoi* has been consumed since pre-Hispanic times and is considered a fundamental ingredient in several dishes, such as the traditional Peruvian ceviche, "picantes" (spicy), and soups [8–12]. However, most of the biomass (60–100% of all *C. chamissoi* exports) is destined for the extraction of carrageenans, polysaccharides with multiple applications in the formulation of various foods due to their binding, emulsifier, and thickener properties [4,13–16]. The remaining exported biomass (at most 40%) is mostly destined for China, Japan, and Taiwan, where it is traditionally used in soups and salads [17]. Thanks to its use as human food and for the hydrocolloid industry, many Peruvian coastal communities (i.e., gatherers and artisanal divers) rely on *C. chamissoi* for their economic livelihood [18,19].

In the last decades, the increasing demand for *C. chamissoi* has decreased populations, along with size reduction and lower gel quality [14]. Moreover, exports have declined during the last five years, while prices have increased sharply since 2019 [17]. This trend is also present in the internal market, where prices experienced an average increase of 366% from 2005 to 2014 [19]. In this context, cultivation efforts have increased during the last few years to achieve sustainable production of this resource [20,21]. Spore-based methods have been applied since 1998; however, they still present several limitations, such as: (1) a high mortality rate of spores during the stages of development; and (2) high operation and labor costs due to long periods of cultivation in controlled conditions (3 to 4 months) [22]. Therefore, an alternative method was proposed by Bulboa et al. [23] based on the formation of secondary attachment discs (SADs) when *C. chamissoi* thalli are anchored on a natural or artificial substratum. This new strategy for vegetative propagation presents several advantages over spore strategies: (1) lower mortality rate; (2) lower operation and labor cost due to shorter incubation time in controlled conditions (3 to 4 weeks); (3) reusability of the thalli, avoiding the massive collection of biomass from natural beds [4,23,24]. The SAD technique has been successfully applied in Chile since its first report [25,26]. In contrast, published cultivation experiences using this approach are still scarce in Peru [27]. It is worth noticing, however, that extensive use of vegetative propagation methods can lead to vigor loss, reduced production capacity, and increased susceptibility to pathogens. Thus, these methods must be combined with reproductive cell (spores or gametes)-based techniques to improve economic cultivars like *C. chamissoi* [28].

Factors such as reproductive phase, seasonality, substrate type, and seawater exchange have been evaluated in *C. chamissoi* vegetative cultures by SAD formation [4,23–25]. Biomass loss during cultivation and/or maintenance periods is critical in seaweed cultures and is attributed to excessive handling, fouling by microscopic organisms, and inadequate culture conditions [29–32]. Our research group has recorded a biomass loss of 20–35% in *C. chamissoi* prior to inoculation for SAD development [33]. However, as far as the authors are aware, suitable conditions for maintenance have not been determined for *C. chamissoi*. Another important factor is the locality (i.e., the sampling site of seaweed inoculum), which is relevant for future algal cultivators to know where to collect robust thalli to generate SADs. To the best of our knowledge, the effect of locality on SAD formation has not been assessed in this commercial red alga. Thus, this work aimed to determine the best conditions for reducing biomass loss in *C. chamissoi* fronds prior to the formation of SADs and to evaluate the effect of locality on SAD development.

2. Results

2.1. Experiment 1: Conditions for Thalli Maintenance Prior Inoculation

BLR (%) ranged from −8.13 to 100% after four weeks in culture. Treatment with fertilized seawater, medium exchange every two days, and inoculum density of 5 g L^{-1} resulted in the lowest biomass loss (−8.13 ± 4.13%), followed by that obtained with fertilized seawater, medium exchange weekly, and inoculum density of 5 g L^{-1}; 0.60 ± 14.44%) and treatment consisting of fertilized seawater, medium exchange weekly, and inoculum density of 3 g L^{-1}; 1.11 ± 11.09%). Fertilization of seawater, medium exchange, and their interaction significantly affected BLR ($p < 0.0001$). The largest effect size was reported for the fertilization of seawater ($\omega^2 = 0.86$). Inoculum density did not affect the BLR ($p = 0.080$) (Table 1). A weekly evaluation of BLR showed that treatments using fertilized seawater did not show significant variations throughout the experiment, i.e., most of the biomass loss in these treatments was reported during the first week. A similar pattern was found in treatments using unfertilized seawater, with some exceptions where BLR increased significantly from the second week onward (Table 2). Overall, loss of biomass could be reduced to 9.36 ± 13.54% and 11.14 ± 14.27% by exchanging the medium (fertilized seawater) every two days or weekly, respectively, regardless of the inoculum density (3–7 g L^{-1}) (Figure 1).

Table 1. Results of a three-way ANOVA evaluating the effect of fertilization with seawater, medium exchange, and inoculum density on the biomass loss rate of *Chondracanthus chamissoi* after four weeks in culture.

Effects	df *	*F* *	*p* *	ω^2 *
Fertilization of seawater (A)	1	232.94	<0.0001	0.86
Medium exchange (B)	2	15.90	<0.0001	0.43
Inoculum density (C)	2	2.71	0.080	NS
A × B	2	13.70	<0.0001	0.39
A × C	2	1.46	0.25	NS
B × C	4	1.61	0.19	NS
A × B × C	4	1.66	0.18	NS

df * = degrees of freedom. *F* * = F statistic. *p* * = significance level. ω^2 * = omega squared (effect size). NS = not significant.

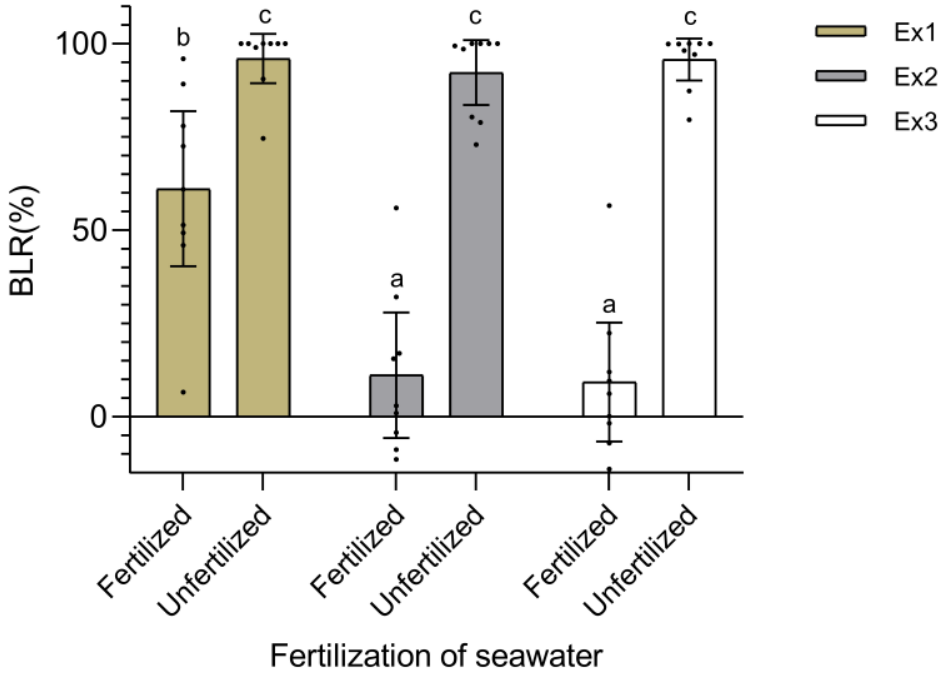


Figure 1. Biomass loss rate (BLR, %) of *Chondracanthus chamissoi* fronds at the end of the maintenance period (4 weeks) using fertilized and unfertilized seawater (Bayfolan® at a final concentration of 0.2 mL L^{-1}) under different conditions of medium exchange (Ex). Values of BLR (%) from different inoculum densities were pooled as this factor was not significant. Lowercase letters indicate significant differences ($p < 0.01$). Independent data points are shown. Error bars represent 95% confidence intervals. Ex1 = daily. Ex2 = every two days. Ex3 = weekly.

A morphological assessment of the fronds evidenced changes in all the treatments. Initially, the fragments had thalli with smooth edges and pointed pinnules and apices (Figure 2a); however, by the second week in culture, the healing process of the fronds and the growth of new shoots (1 to 2 mm in length) on the edges of the thalli and pinnules were evident (Figure 2b). By the fourth week of culture, the pinnules and apices began to show elongated and pointed shapes, narrowing and curving (Figure 2c).

Table 2. Weekly biomass loss rate (BLR, %) of *Chondracanthus chamissoi* fronds under different treatments for maintenance prior to inoculation and secondary attachment disc (SAD) formation. Superscript letters indicate significant differences ($p < 0.01$) among weeks for each treatment. Values are presented as the mean ± 95% confidence interval ($n = 3$).

Fertilization of Seawater *	Medium Exchange **	Inoculum Density (g L^{-1})	Week 1	Week 2	Week 3	Week 4
Fertilized	Daily (Ex1)	3	3.44 ± 15.24% ^{a}	14.56 ± 18.08% ^{a}	54.89 ± 23.38% ^{a}	74.44 ± 26.64% ^{a}
		5	1.40 ± 11.30% ^{a}	19.07 ± 15.32% ^{a}	45.67 ± 44.57% ^{a}	56.13 ± 49.44% ^{a}
		7	−1.10 ± 4.25% ^{a}	8.85 ± 7.54% ^{a}	26.86 ± 4.20% ^{a}	52.81 ± 8.59% ^{a}
	Every two days (Ex2)	3	−2.89 ± 4.17% ^{a}	−1.56 ± 3.30% ^{a}	−5.00 ± 6.63% ^{a}	7.00 ± 9.86% ^{a}
		5	−4.20 ± 4.19% ^{a}	−4.73 ± 2.44% ^{a}	−11.80 ± 2.55% ^{a}	−8.13 ± 4.12% ^{a}
		7	19.71 ± 21.40% ^{a}	31.90 ± 23.71% ^{a}	30.71 ± 24.62% ^{a}	34.57 ± 23.00% ^{a}
	Weekly (Ex3)	3	−4.00 ± 8.91% ^{a}	−2.44 ± 11.22% ^{a}	−7.22 ± 14.98% ^{a}	1.11 ± 11.09% ^{a}
		5	−8.67 ± 3.40% ^{a}	−1.93 ± 12.03% ^{a}	−2.33 ± 10.24% ^{a}	0.60 ± 14.44% ^{a}
		7	21.29 ± 28.31% ^{a}	27.38 ± 30.44% ^{a}	23.67 ± 32.58% ^{a}	26.38 ± 32.16% ^{a}
Unfertilized	Daily (Ex1)	3	13.67 ± 11.51% ^{a}	67.89 ± 35.67% ^{a}	81.89 ± 29.50% ^{a}	91.22 ± 16.23% ^{a}
		5	7.40 ± 20.77% ^{a}	80.47 ± 31.14% ^{a}	92.00 ± 14.71% ^{a}	96.87 ± 6.15% ^{a}
		7	2.29 ± 9.54% ^{a}	97.14 ± 1.54% ^{b}	99.71 ± 0.43% ^{b}	100% ^{b}
	Every two days (Ex2)	3	1.44 ± 4.29% ^{a}	78.89 ± 26.06% ^{ab}	99.78 ± 0.43% ^{b}	100% ^{b}
		5	2.13 ± 4.19% ^{a}	12.47 ± 7.63% ^{a}	48.00 ± 22.24% ^{a}	84.00 ± 14.91% ^{a}
		7	3.52 ± 2.18% ^{a}	38.14 ± 52.81% ^{ab}	82.72 ± 33.75% ^{ab}	92.76 ± 13.63% ^{b}
	Weekly (Ex3)	3	10.88 ± 18.89% ^{a}	59.11 ± 7.55% ^{a}	70.22 ± 17.52% ^{a}	88.99 ± 11.60% ^{a}
		5	−2.66 ± 3.76% ^{a}	95.00 ± 9.80% ^{b}	98.33 ± 3.27% ^{b}	99.99 ± 0.01% ^{b}
		7	−6.42 ± 11.83% ^{a}	93.76 ± 2.73% ^{b}	96.19 ± 2.29% ^{b}	98.43 ± 1.64% ^{b}

* Seawater was fertilized using Bayfolan® at a final concentration of 0.2 mL L^{-1}. ** All the seawater was removed in each exchange.



Figure 2. Morphological variation of *Chondracanthus chamissoi* fragments during the maintenance process in semi-controlled laboratory conditions. (**a**) Fronds at the early stage of cultivation. (**b**) Fronds after two weeks of maintenance. Note the increase in pinnules and small shoots over the entire surface of the fragment. (**c**). Fronds after four weeks of maintenance. Note the change in quantity, shape, and size of the pinnules and shoots over the entire surface of the fragment. All figures shown are on a scale of 5 cm.

2.2. Experiment 2: Effect of Locality on SAD Formation

All treatments showed SAD formation after 25 days of culture (Figure 3a,b). The number of SADs per shell ranged from 7 to 17. Treatment MVI (vegetative fronds from the

intertidal zone in Mendieta) presented the highest formation of SADs (17 ± 2 SADs shell^{-1}), followed by that obtained in treatment TVS (vegetative fronds from the subtidal zone in Talpo; 15 ± 4 SADs shell^{-1}) and treatment PrVS (vegetative fronds from the subtidal zone in Punta Ripio; 15 ± 2 SADs shell^{-1}). Although there was a tendency towards higher SAD values in treatments using vegetative thalli, only intertidal vegetative fronds from Mendieta (treatment MVI) showed statistically higher values (17 ± 2 SADs shell^{-1}) compared to cystocarpic plants from the same zone (treatment MCI; 7 ± 1 SADs shell^{-1}; $p = 0.0052$). Most of the localities showed similar values of SAD formation regardless of the reproductive state of the material and the collection zone (intertidal vs. subtidal). Only intertidal vegetative plants from Mendieta (treatment MVI) showed superior SAD formation compared to subtidal vegetative plants from Tanque Amarillo (treatment TaVS; 11 ± 1 SADs shell^{-1}; $p < 0.0001$) (Figure 3a).

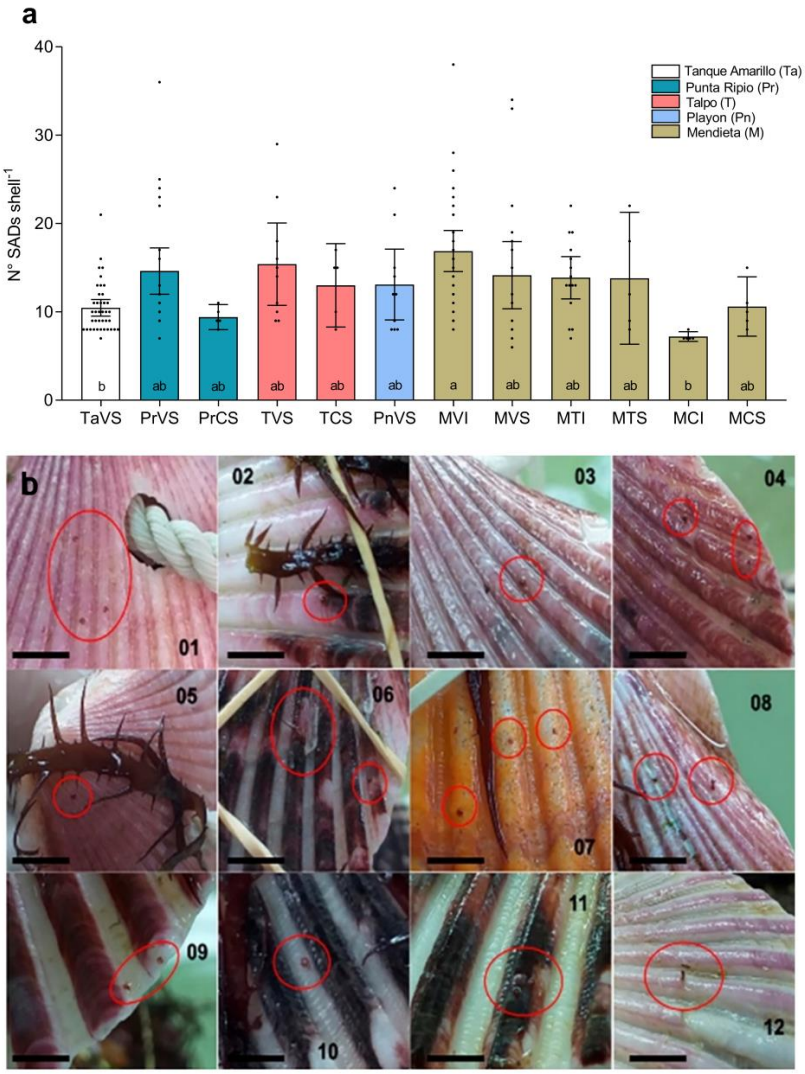


Figure 3. Secondary attachment disc (SAD) formation by *Chondracanthus chamissoi* in five localities. (**a**) Average number of SADs per shell. The reproductive stages considered were cystocarpic (C), tetrasporophytic (I), and vegetative (V). Most of the plants were collected from the subtidal zones (S). In the case of Mendieta, plants from the intertidal zone (I) were also used. (**b**) Representative pictures of SADs (red circles) are shown for Mendieta (01, 02, 03, 04, 05, 06), Punta Ripio (07, 08, 09), Talpo (10, 11), and Playon (12). All figures shown in (**b**) are on a scale of 1 cm. Lowercase letters in (**a**) indicate significant differences ($p < 0.01$). Independent data points are shown. Error bars represent 95% confidence intervals.

The qualitative assessment showed that more than half of vegetative plants from Mendieta (treatments MVI and MVS; 54.28–62.59%) and Punta Ripio (60%; PrVS) presented the lowest level of necrosis and deterioration (<25% of the frond). Among the evaluated localities, Mendieta showed the smallest values, with 54.28–62.59% of the plants showing <25% of the frond with signs of necrosis/deterioration. Within each locality, necrosis and deterioration were usually higher in tetrasporophytic and cystocarpic plants (>25% of the frond) than in vegetative ones (<25% of the frond) (Figure 4).

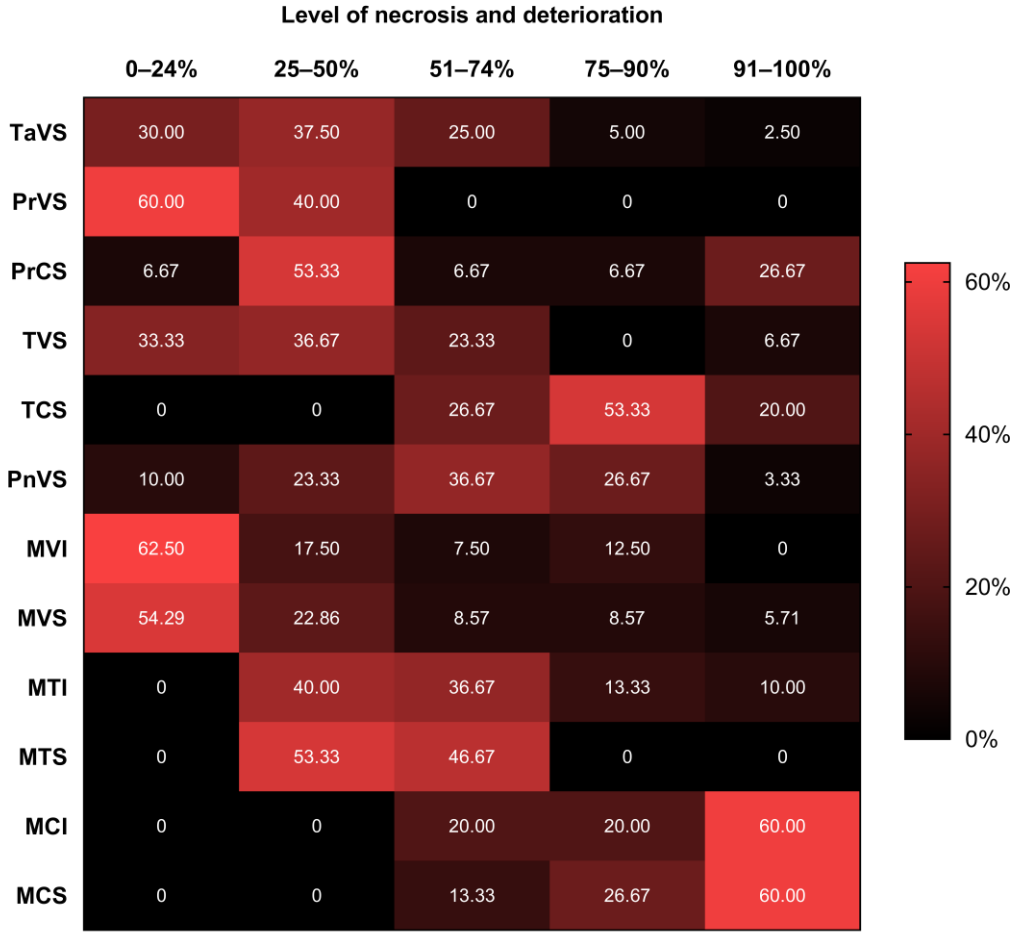


Figure 4. Heat map showing the percentage of fronds with different levels of necrosis and deterioration in *Chondracanthus chamissoi* at the end of secondary attachment disc (SAD) experiments (25 days). Five localities were evaluated: Tanque Amarillo (Ta), Punta Ripio (Pr), Talpo (T), Playon (Pn), and Mendieta (M). The reproductive stages considered were cystocarpic (C), tetrasporophytic (I), and vegetative (V). Most of the plants were collected from the subtidal zones (S). In the case of Mendieta, plants from the intertidal zone (I) were also used.

3. Discussion

The physiological requirements of a seaweed cultivar and the laboratory protocols for its maintenance are pivotal for a successful culture [34,35]. The low availability of nutrients (especially nitrogen) limits macroalgae growth. Therefore, these elements are usually incorporated into closed cultivation systems of commercially valuable seaweeds to improve growth [36–39]. One way to incorporate nutrients is by adding agricultural fertilizers, which allow for high yields at a low cost. Various species have been cultivated by adding agricultural fertilizers: *Gracilaria chilensis* [40], *Chondracanthus squarrulosus* [41], *Porphyra* spp. [42,43], *Sarcothalia crispata* [44], *Sarcopeltis skottsbergii* (formerly *Gigartina skottsbergii*) [45,46], and *Chondracanthus chamissoi* [20,47]. Likewise, the commercial fertilizer Bayfolan® (Bayer, Lima, Peru) has been widely used in macroalgae cultures, demonstrating

good performance [20,43,46]. This liquid fertilizer has a concentrated nutrient formula, including vitamins and indoleacetic acid, which stimulate plant growth and could have the same effect on algae [48]. In our experiments, the use of Bayfolan® with a medium exchange every two days or weekly resulted in the lowest biomass loss for *C. chamissoi* fronds, regardless of the inoculum density. In this regard, high levels of biomass loss in treatments without fertilization may be associated with a limitation of essential nutrients for seaweed metabolism [49].

Water flow has an important role in the uptake of nutrients, with low-speed flows resulting in less nutrient uptake [36,49,50]. In general, it is considered that while the flow or exchange of the culture medium is higher, the maintenance of inoculums and growth will be favored by a better uptake of nutrients [51–53]. For example, Bulboa et al. [23] demonstrated a greater formation and growth of SADs in *C. chamissoi* treatments with a constant flow of seawater (open flow). Similarly, Grote [54] identified the need to carry out regular water exchanges to have greater availability of nutrients for the cultivation of *Palmaria palmata*. The author pointed out that higher yields were obtained with exchange rates greater than six per day. Nevertheless, our data showed that a high frequency of medium exchange (daily) was detrimental to *C. chamissoi* maintenance. This may be due to a higher manipulation of *C. chamissoi* fragments, causing greater stress on the algae, which affected the healing process and ultimately caused biomass loss [29]. Furthermore, the physicochemical conditions of the culture medium could have been more stable in treatments with a lower frequency of medium exchange (two times a week or weekly), reducing the stress conditions. High inoculum densities can also negatively impact seaweed growth and development by reducing the availability of light, nutrients, and/or substrate [36,53,55–58]. This factor did not significantly affect the biomass loss rate in our experiments. However, we do not discard the fact that higher densities than those evaluated in this work might negatively affect *C. chamissoi* maintenance.

Besides the production of gametes and spores (tetraspores or carpospores), several studies have determined the ability of free fragments of *C. chamissoi* to re-attach to a substrate, forming basal crustose systems with the capacity to generate new fronds [59–62]. This process explains the morphological variation observed in *C. chamissoi* throughout the maintenance period, which facilitates the re-attachment by forming newly elongated, pointed, and curved pinnules and shoots [63].

Our work also demonstrated the ability of all *C. chamissoi* reproductive phases (i.e., tetrasporophyte, cystocarpic, and vegetative) from the five localities (Pisco, Peru) to propagate vegetatively by means of SAD formation on a natural substrate (*Argopecten purpuratus* shells). Similarly, Zapata-Rojas et al. [27] could induce SAD formation in two other natural substrates (clam and South Pacific abalone shells) using biomass collected in southern Peru (Moquegua region). However, the authors did not quantify the number of SAD per shell, and thus we could not compare our results with theirs. It is worth noticing that, in our work, all SADs came from vegetative propagation, as they were produced from the contact points between the inoculum (mother algae) and the substrate. No seedlings from a sporulation process were observed in any treatment. Vegetative fronds from the intertidal zone of Mendieta showed the highest SAD formation. Individuals from this locality presented suitable morphological features, i.e., two or more thick main axes and pointed apices with abundant lateral branches [47]. These characteristics would allow fronds to cover the substrate more efficiently and to receive a uniform nutrient flow. Conversely, among vegetative individuals, the ones from Tanque Amarillo showed the lowest number of SADs per shell. Their morphology, i.e., thick stems and branches (6–10 mm) with scarce secondary branches or pinnules, did not favor SAD formation. Overall, most localities showed a similar capacity to produce SADs (7–17 SADs shell^{-1}). As far as the authors are aware, there are no references in the literature assessing the effect of locality on the vegetative propagation of *C. chamissoi* via SADs. According to Véliz et al. [34], the photosynthetic characteristics, pigment concentrations, antioxidant capacity, and MAA contents of *C. chamissoi* varied among populations along its distributional range along the Chilean

coast, suggesting an ecotypic differentiation in this species. However, it is not clear whether this affects SAD formation in *C. chamissoi*. Localities from a wider geographical area in Peru and Chile must be included in future cultivation experiments to clarify this effect.

Regarding the reproductive phase, Bulboa et al. [23] determined that the most suitable conditions for vegetation propagation of *C. chamissoi* via SAD formation involved the use of individuals without obvious reproductive structures. This is because seaweeds allocate between 40% and 50% of the annual production of their biomass to their reproductive effort [64]. Our results confirmed this tendency only in fronds from Mendieta. Thus, locality might also be affecting the SAD formation capacity of *C. chamissoi*. In addition, the intertidal habit might affect SAD formation as individuals are naturally adapted to grow on wide rocky surfaces, forming more lateral outgrowths. Further experiments involving more localities and individuals from all reproductive phases and zones are needed to shed light on the effects of these two factors.

Necrosis and deterioration of the fronds (mainly in the apices and portions of the thallus pressed by the elastic bands) were observed in all treatments to a greater or lesser extent as a consequence of lacking an adaptation period or acclimatization. This was more critical in cystocarpic algae, as they presented irregular thallus surfaces due to the presence of reproductive structures. This feature makes the algae more prone to injuries that could become infected, generating tissue loss via necrosis. Furthermore, the irregular surface might cause "dead areas" with inadequate nutrient flow and light penetration. On the contrary, necrosis and deterioration of vegetative algae remained at acceptable levels at the end of the experiment, i.e., only 0–25% of the frond surfaces were damaged. This was more evident in intertidal vegetative fronds from Mendieta, probably because intertidal algae are more tolerant to stress than subtidal algae [65]. Some authors have found lower growth and survival rates in reproductive specimens (cystocarpic or tetrasporophytic) compared to non-reproductive (vegetative) individuals [66,67]. Furthermore, Guillemin et al. [68] reported a slower growth rate and higher mortality in reproductive fronds of *Gracilaria chilensis* compared to vegetative ones. These differences were related to lower pigment concentrations and net productivity (metabolic rates and primary productivity).

4. Materials and Methods

4.1. Sampling Sites

Chondracanthus chamissoi fronds were obtained by semi-autonomous diving in subtidal natural beds (2.5–4 m in depth) at Tanque Amarillo (13°46.73′ S; 76°14.38′ W), Punta Ripio (13°47.78′ S; 76°17.65′ W), Talpo (13°48.09′ S; 76°20.74′ W), Playon (14°1.56′ S; 76°15.78′ W), and Mendieta (14°3.30′ S; 76°15.69′ W) (Figure 5). Abiotic parameters in the area were 16–19 °C, 5–6.5 mg O_2 L^{-1}, pH of 7.1–8.1, and salinity of 36.3–36.8 g kg^{-1}. Fronds were transported in ice boxes at 5–10 °C to the Laboratorio de Investigación en Cultivos Marinos (LICMA) of the Universidad Científica del Sur (13°43′49.9″ S; 76°13′24.2″ W), where the experiments were carried out between March and August 2019. Specimens were cleaned as described by Macchiavello et al. [25].

The selected sites are located in the Ica region, on the south-central coast of Peru, a zone that is one of the most productive and commercial areas in the country, comprising more than ten *C. chamissoi* natural beds that are exploited throughout the year [69,70].

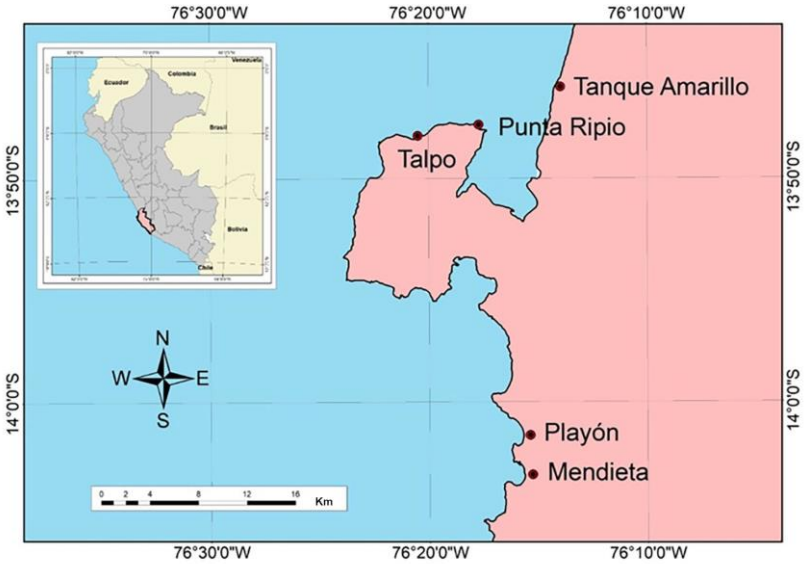


Figure 5. Map of sampling sites for *Chondracanthus chamissoi* (Ica, Peru).

4.2. Experiment 1

The effect of fertilization of seawater (with Bayfolan® at a final concentration of 0.2 mL L^{-1}), medium exchange, and inoculum density on biomass loss rate (BLR, %) was assessed in a multifactorial experiment with three repetitions per treatment. Factor levels and conditions are shown in Table 2. Vegetative thalli (20 kg fresh weight) from Mendieta were cut into 5 ± 1 cm fragments and transferred to 1-L transparent plastic containers with seawater disinfected with sodium hypochlorite. Culture conditions were 18.5 °C, constant aeration, a 12:12-h light/dark photoperiod, and a light intensity of 30 µmol photons m^{-2} s^{-1}. BLR (%) was calculated using the following formula:

$$BLR(\%) = \left(\frac{W_{t_0} - W_{t_n}}{W_{t_0}}\right) * 100$$

where W_{t_0} is the initial fresh weight; and W_{t_n} is the fresh weight after "n" weeks. BLR (%) was assessed weekly for four weeks.

4.3. Experiment 2

The effect of locality on the number of SADs was assessed in a unifactorial experiment (Table 3). Fronds (10 kg fresh weight) from the five abovementioned localities with 10–16 cm in length were used. In the case of Mendieta, specimens from intertidal beds were also collected. The following morphological features were considered during the selection of individuals: (a) a main axis of 4–7 mm in width with a pointed apex and thick pinnules; and/or (b) one or several erect axes with subdichotomous branches of 2–4 mm in width, abundant secondary branches and pinnules, and pointed apexes. The thalli were further separated into cystocarpic (female gametophytes), tetrasporophytic, and vegetative thalli. It is worth noticing that not all the localities presented individuals in these three reproductive phases showing suitable morphological features for SAD formation.

The cultivation method proposed by Bulboa et al. [23] was used in this experiment with some modifications. Scallop shells (8–10 cm in length) were used as a natural substratum. The shells were cleaned by submerging them in distilled water with sodium hypochlorite (0.2%) for 3 h to remove fouling and disinfect them, and then they were rinsed with distilled water. Each shell was perforated and arranged in a set of five shells with a 10 cm separation from each other. They were fixed by knots to 120 cm long ropes. This set was fixed to a PVC structure of 140 cm in length that was placed over 2.3 cm^3 tanks containing 10 µm of filtered seawater. Two tanks were used for all treatments. *C. chamissoi* fronds were fixed over each shell by an elastic band (Figure 6). The number of experimental units inoculated (shells with

fronds) was directly proportional to the amount of biomass available for each treatment. Culture conditions were 20 °C, constant aeration, under a 16:8-h light/dark photoperiod, with a light intensity of 25–30 µmol photons m^{-2} s^{-1}, and a salinity of 36–37 g kg^{-1}. After 25 days of cultivation, fronds were removed from the shells, and the number of SADs per shell was determined. Additionally, an arbitrary five-level scale was used to assess the necrosis and deterioration of the thalli at the end of the experiment (Figure 7). The percentage of fronds in each treatment was classified according to this scale.

Table 3. Treatments used for secondary attachment disc (SAD) formation in *Chondracanthus chamissoi*.

Locality	Reproductive Stage	Zone	Treatment
Tanque Amarillo	Vegetative	Subtidal	TaVS
Punta Ripio	Vegetative	Subtidal	PrVS
	Cystocarpic	Subtidal	PrCS
Talpo	Vegetative	Subtidal	TVS
	Cystocarpic	Subtidal	TCS
Playon	Vegetative	Subtidal	PnVS
Mendieta	Vegetative	Intertidal	MVI
	Vegetative	Subtidal	MVS
	Tetrasporophytic	Intertidal	MTI
	Tetrasporophytic	Subtidal	MTS
	Cystocarpic	Intertidal	MCI
	Cystocarpic	Subtidal	MCS

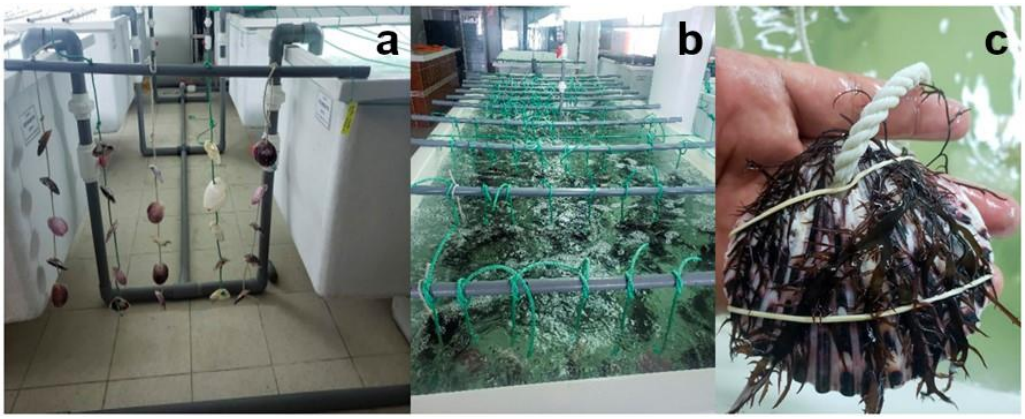


Figure 6. Culture system for secondary attachment disc (SAD) formation by *Chondracanthus chamissoi*. (**a**) Natural substrata (scallop shells) fixed by knots to 120 cm long ropes and anchored to a PVC structure of 140 cm in length. (**b**) Culture tanks. (**c**) *C. chamissoi* frond fixed over a shell by an elastic band.

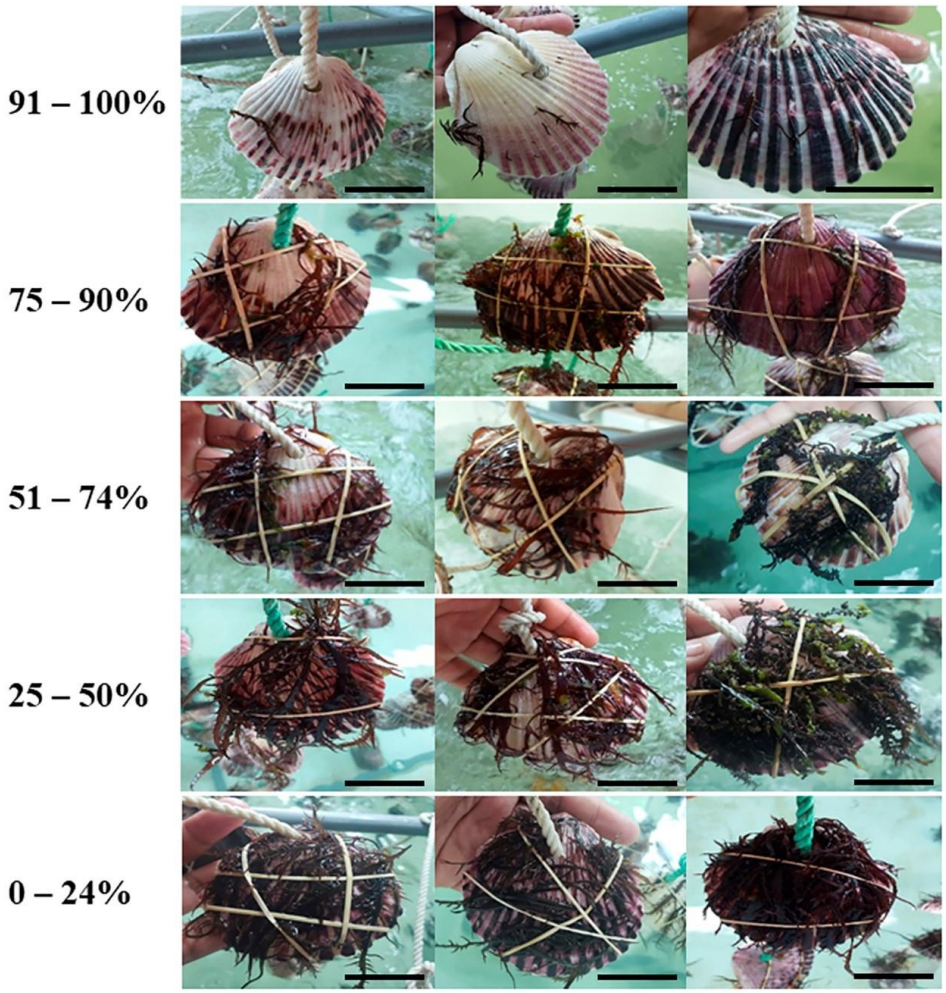


Figure 7. A five-level scale used for qualitative assessment of necrosis and deterioration in *Chondracanthus chamissoi* fronds at the end of secondary attachment disc (SAD) development experiments (25 days). All figures shown are on a scale of 5 cm.

4.4. Statistical Analyses

The effect of time (weeks) in BLR under different treatments for maintenance prior inoculation and secondary attachment disc (SAD) formation was assessed using repeated-measures ANOVA. The Bonferroni method was chosen for multiple comparisons. A three-way analysis of variance (ANOVA) was used to compare BLR under conditions of fertilization with seawater, medium exchange, and inoculum density. Normality and homoscedasticity were examined using residual plots and Levene, respectively, prior to the conduction of parametric tests. A Sidak post-hoc test was used when the results were significant. Effect size [71] was presented as ω^2 [72] in the case of the obtaining of significant factors. The analyses were performed using the "car" [73] and "multcomp" [74] packages in R v. 4.1.2.

The number of SADs was analyzed using either a negative binomial or Poisson regression model. A likelihood ratio test was used to decide which count regression model to use. The analyses were performed using the "pscl" [75,76] and "MASS" [77] packages in R v.4.1.2.

The significance threshold was set at $p = 0.01$ to reduce the true Type I error rate [78]. All graphs were created using GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA).

5. Conclusions

Fertilized seawater with Bayfolan® (0.2 mL L^{-1}) and a medium exchange every two days or weekly were the best conditions for the maintenance of *C. chamissoi* fronds prior to vegetative propagation, regardless of the inoculum density. SAD formation was successfully induced in fronds from all the localities, reproductive phases, and zones tested. In general, most of the localities showed a similar capacity to produce SADs (7–17 SADs $shell^{-1}$). However, there was a tendency for vegetative algae, especially Mendieta, to present higher SADs per shell. Vegetative fronds also showed lower levels of necrosis and deterioration compared to cystocarpic and tetrasporophytic samples. This was more noticeable in vegetative intertidal fronds from Mendieta. Thus, among the localities tested, we recommend *C. chamissoi* vegetative fronds from the intertidal zone of Mendieta as the best source of biomass for vegetative propagation via SAD formation. This technique might represent a viable methodology for developing repopulation and/or intensive cultivation projects in Peru.

Author Contributions: Conceptualization, S.A., M.C.-F., A.M.-R. and P.B.; methodology, S.A., M.C.-F., A.M.-R., O.A. and P.B.; software, J.A.-P. and O.A.; validation, J.A.-P.; formal analysis, J.A.-P. and O.A.; investigation, S.A., J.A.-P., M.C.-F., A.M.-R. and P.B.; resources, S.A., M.C.-F., A.M.-R. and P.B.; data curation, J.A.-P.; writing—original draft preparation, J.A.-P.; writing—review and editing, J.A.-P. and P.B.; visualization, S.A. and J.A.-P.; supervision, P.B.; project administration, P.B.; funding acquisition, P.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Programa Nacional de Innovación en Pesca y Acuicultura (PNIPA), Nº 136-2018-PNIPA-Subproyectos "Implementation of a commercial pilot cultivation of *Chondracanthus chamissoi* by vegetative propagation in the Paracas Bay", and Universidad Científica del Sur (UCSUR Nº 061-2022-PRO999).

Data Availability Statement: The data that support the findings of this study are available from the corresponding author, Paul Baltazar, upon reasonable request.

Acknowledgments: The authors thank Cooperativa de Trabajadores Pesqueros Artesanales Algas Marinas (COTRAPALMAR) for its support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Arakaki, N.; Carbajal, P.; Marquez-Corigliano, D.; Suárez Alarcón, S.; Gil-Kodaka, P.; Perez-Araneda, K.; Tellier, F. Genética de macroalgas en el Perú: Diagnóstico, guía metodológica y casos de estudio. *Inf. Inst. Mar. Perú* **2021**, *48*, 594–609.
2. Yang, M.Y.; Macaya, E.C.; Kim, M.S. Molecular evidence for verifying the distribution of *Chondracanthus chamissoi* and *C. teedei* (Gigartinaceae, Rhodophyta). *Bot. Mar.* **2015**, *58*, 103–113. [CrossRef]
3. Arakaki, N.; Suárez-Alarcón, S.; Márquez-Corigliano, D.; Gil-Kodaka, P.; Tellier, F. The widely distributed, edible seaweeds in Peru, *Chondracanthus chamissoi* and *Chondracanthus chamissoi* f. glomeratus (Gigartinaceae, Rhodophyta), are morphologically diverse but not phylogenetically distinct. *J. World Aquac. Soc.* **2021**, *52*, 1290–1311. [CrossRef]
4. Bulboa, C.; Macchiavello, J. Cultivo de frondas cistocárpicas, tetraspóricas y vegetativas de *Chondracanthus chamissoi* (Rhodophyta, Gigartinales) en dos localidades del norte de Chile. *Investig. Mar.* **2006**, *34*, 109–112. [CrossRef]
5. Calderón, M.; Ramírez, M.E.; Bustamante, D. Notas sobre tres especies de Gigartinaceae (Rhodophyta) del litoral peruano. *Rev. Peru. Biol.* **2010**, *17*, 115–122. [CrossRef]
6. Rodríguez, C.Y.; Tellier, F.; Pérez-Araneda, K.; Otaíza, R.D. Taxonomic position of the two sympatric forms of *Chondracanthus chamissoi* (f. lessonii and f. chauvinii) (Rhodophyta, Gigartinaceae) by using two molecular markers. *Lat. Am. J. Aquat. Res.* **2021**, *49*, 182–187. [CrossRef]
7. Guiry, M.D.; Guiry, G.M. *AlgaeBase. World-Wide Electronic Publication*; National University of Ireland: Galway, Ireland; Available online: http://www.algaebase.org (accessed on 5 April 2023).
8. Acosta, J. *Nombres Vulgares y Usos de las Algas en el Perú*; Departamento de Botánica, Museo de Historia Natural Javier Prado: Jesús María, Perú, 1977; Volume 7, pp. 1–9.
9. Donnan, C.B.; McClelland, D. Moche burials at Pacatnamu. In *The Pacatnamu Papers. The Moche Occupation*; Donnan, C.B., Cock, G.A., Eds.; Fowler Museum of Cultural History, University of California: Los Angeles, CA, USA, 1997; Volume 2, pp. 17–187.
10. Noriega, C. *Algas Comestibles del Perú. Pan del Futuro*; Universidad San Martín de Porres: Lima, Peru, 2010; pp. 1–180.
11. Ugás, R. *40 Verduras Viejas y Nuevas Para Diversificar Tu Alimentación y Nutrirte Mejor*; Universidad Nacional Agraria La Molina: Lima, Peru, 2014; pp. 1–116.

12. Bonavia, D.; Vasquez, V.F.; Rosales Tham, T.; Dillehay, T.D.; Netherly, P.J.; Benson, K. Plant remains. In *Where the Land Meets the Sea: Fourteen Millennia of Human History at Huaca Prieta, Peru*; Dillehay, T.D., Ed.; University of Texas Press: Austin, TX, USA, 2017; pp. 367–433.
13. Icochea, E. Bases biológicas para el Manejo del Recurso *Chondracanthus chamissoi* en el Litoral Marino de Huanchaco, Departamento La Libertad, Perú. Master's Thesis, Universidad Nacional de Trujillo, Trujillo, Peru, 2008.
14. Hayashi, L.; Bulboa, C.; Kradolfer, P.; Soriano, G.; Robledo, D. Cultivation of red seaweeds: A Latin American perspective. *J. Appl. Phycol.* **2014**, *26*, 719–727. [CrossRef]
15. Alemañ, A.E.; Robledo, D.; Hayashi, L. Development of seaweed cultivation in Latin America: Current trends and future prospects. *Phycologia* **2019**, *58*, 462–471. [CrossRef]
16. Avila-Peltroche, J.; Padilla-Vallejos, J. The seaweed resources of Peru. *Bot. Mar.* **2020**, *62*, 381–394. [CrossRef]
17. Avila-Peltroche, J.; Villena-Sarmiento, G. Analysis of Peruvian seaweed exports during the period 1995–2020 using trade data. *Bot. Mar.* **2022**, *65*, 209–220. [CrossRef]
18. Acleto, C. *Algas Marinas del Perú de Importancia Económica*; Departamento de Botánica, Museo de Historia Natural Javier Prado: Jesús María, Perú, 1986; Volume 5, pp. 1–107.
19. Diaz Ruíz, J.T.; Fretell Timoteo, W.J.; Baltazar Guerrero, P.M.; Castañeda Franco, M.; Meza Balvin, S.J.; Ordoñez Suñiga, C.A. Factibilidad económica de la producción de *Chondracanthus chamissoi*, cultivo vía esporas en laboratorio, San Andrés-Pisco, Perú. *Arnaldoa* **2021**, *28*, 163–182.
20. Castañeda, M.; Arbaiza, S.; Diaz, F.; Castillo, Y.; Baltazar, P.; Advíncula, O. Evaluación del fotoperiodo en el asentamiento de tetraesporas de *Chondracanthus chamissoi* sobre cuerdas de polipropileno en condiciones semi-controladas de laboratorio. *Anales Científicos* **2018**, *79*, 459–465. [CrossRef]
21. Arbaiza, S.; Gil-Kodaka, P.; Arakaki, N.; Alveal, K.; Arbaiza, S.; Gil-Kodaka, P.; Arakaki, N.; Alveal, K. Primeros estadios de cultivo a partir de carpósporas de *Chondracanthus chamissoi* de tres localidades de la costa peruana. *Rev. Biol. Mar. Oceanogr.* **2019**, *54*, 204–213. [CrossRef]
22. Bulboa, C. Bases Bio-Tecnológicas para o Cultivo de *Chondracanthus chamissoi*, Uma Alga Vermelha de Importância Econômica da Costa Chilena. Ph.D. Thesis, São Paulo University, São Paulo, Brazil, 2006.
23. Bulboa, C.; Véliz, K.; Sáez, F.; Sepúlveda, C.; Vega, L.; Macchiavello, J. A new method for cultivation of the carragenophyte and edible red seaweed *Chondracanthus chamissoi* based on secondary attachment disc: Development in outdoor tanks. *Aquaculture* **2013**, *410–411*, 86–94. [CrossRef]
24. Sáez, F.; Macchiavello, J. Secondary attachment discs: A new alternative for restoring populations of *Chondracanthus chamissoi* (Gigartinales, Rhodophyta). *Lat. Am. J. Aquat. Res.* **2018**, *46*, 140–146. [CrossRef]
25. Macchiavello, J.; Sepúlveda, C.; Basaure, H.; Sáez, F.; Yañez, D.; Marín, C.; Vega, L. Suspended culture of *Chondracanthus chamissoi* (Rhodophyta: Gigartinales) in Caleta Hornos (northern Chile) via vegetative propagation with secondary attachment discs. *J. Appl. Phycol.* **2018**, *30*, 1149–1155. [CrossRef]
26. Oyarzo, S.; Ávila, M.; Alvear, P.; Remonsellez, J.P.; Contreras-Porcia, L.; Bulboa, C. Secondary attachment disc of edible seaweed *Chondracanthus chamissoi* (Rhodophyta, Gigartinales): Establishment of permanent thalli stock. *Aquaculture* **2021**, *530*, 735954. [CrossRef]
27. Zapata-Rojas, J.C.; Gonzales-Vargas, A.M.; Zevallos-Feria, S.A. Estudio comparativo para propagación vegetativa de *Chondracanthus chamissoi*, Yuyo, sobre tres tipos de sustrato en ambiente controlado y su viabilidad en la región Moquegua. *Enfoque UTE* **2020**, *11*, 37–47. [CrossRef]
28. Jiksing, C.; Ongkudon, M.M.; Thien, V.Y.; Rodrigues, K.F.; Yong, W.T.L. Recent advances in seaweed seedling production: A review of eucheumatoids and other valuable seaweeds. *Algae* **2022**, *37*, 105–121. [CrossRef]
29. Marinho-Soriano, E.; Morales, C.; Moreira, W.S.C. Cultivation of *Gracilaria* (Rhodophyta) in shrimp pond effluents in Brazil. *Aquac. Res.* **2002**, *33*, 1081–1086. [CrossRef]
30. Mai, H.; Fotedar, R.; Fewtrell, J. Evaluation of *Sargassum* sp. as a nutrient-sink in an integrated seaweed-prawn (ISP) culture system. *Aquaculture* **2010**, *310*, 91–98. [CrossRef]
31. Suthar, P.; Gajaria, T.K.; Reddy, C.R.K. Production of quality seaweed biomass through nutrient optimization for the sustainable land-based cultivation. *Algal Res.* **2019**, *42*, 101583. [CrossRef]
32. García-Poza, S.; Leandro, A.; Cotas, C.; Cotas, J.; Marques, J.C.; Pereira, L.; Gonçalves, A.M. The evolution road of seaweed aquaculture: Cultivation technologies and the industry 4.0. *Int. J. Environ. Res. Public Health* **2020**, *17*, 6528. [CrossRef]
33. Baltazar, P. *Personal Communiacation*; Universidad Científica del Sur: Lima, Perú, 2022.
34. Véliz, K.; Chandía, N.; Karsten, U.; Lara, C.; Thiel, M. Geographic variation in biochemical and physiological traits of the red seaweeds *Chondracanthus chamissoi* and *Gelidium lingulatum* from the south east Pacific coast. *J. Appl. Phycol.* **2019**, *31*, 665–682. [CrossRef]
35. Winberg, P.; Skropeta, D.; Ullrich, A. *Seaweed Cultivation Pilot Trials: Towards Culture Systems and Marketable Products*; Australian Government Rural Industries Research and Development Corporation (RIRDC): Canberra, Australia, 2011; pp. 1–184.
36. Hurd, C.L.; Harrison, P.J.; Bischof, K.; Lobban, C.S. *Seaweed Ecology and Physiology*, 2nd ed.; Cambridge University Press: Cambridge, UK, 2014; pp. 1–565.
37. Ferreira, L.B.; Barufi, J.B.; Plastino, E.M. Growth of red and green strains of the tropical agarophyte *Gracilaria cornea* J. Agardh (Gracilariales, Rhodophyta) in laboratory. *Rev. Bras. Bot.* **2006**, *29*, 187–192. [CrossRef]

38. Mansilla, A.; Rodriguez, J.P.; Souza, J.M.; Rosenfeld, S.; Ojeda, J.; Yokoya, N.S. Growth responses to temperature, salinity and nutrient variations, and biomass variation and phenology of *Ahnfeltia plicata* (Rhodophyta, Ahnfeltiales): A commercially interesting agarophyte from the Magellanic Region, Chile. *J. Appl. Phycol.* **2014**, *26*, 1133–1139. [CrossRef]
39. Yong, W.T.L.; Ting, S.H.; Yong, Y.S.; Thien, V.Y.; Wong, S.H.; Chin, W.L.; Anton, A. Optimization of culture conditions for the direct regeneration of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae). *J. Appl. Phycol.* **2014**, *26*, 1597–1606. [CrossRef]
40. Alveal, K.; Romo, H.; Werlinger, C.; Oliveira, E. Mass cultivation of the agar-producing alga *Gracilaria chilensis* (Rhodophyta) from spores. *Aquaculture* **1997**, *148*, 77–83. [CrossRef]
41. Pacheco-Ruiz, I.; Zertuche-González, J.A.; Arroyo-Ortega, E.; Valenzuela-Espinoza, E. Agricultural fertilizers as alternative culture media for biomass production of *Chondracanthus squarrulosus* (Rhodophyta, Gigartinales) under semi-controlled conditions. *Aquaculture* **2004**, *240*, 201–209. [CrossRef]
42. Romo, H.; Ávila, M.; Candía, A.; Nuñez, M.; Oyarzo, C.; Gallegillos, F.; Cáceres, J. *Manual de Técnicas de Cultivo de Luche (Porphyra sp.)*; Proyecto FONDEF D01 I 1148; IFOP: New York, NY, USA, 2005; pp. 1–32.
43. Arbaiza, S.; Castañeda, M.; Gerónimo, G.; Munayco, P.; Reynaga, R.; Advíncula, O. Efecto del fotoperiodo y nutriente foliar comercial en el crecimiento (biomasa) de cochayuyo Porphyra spp. bajo condiciones semicontroladas de cultivo. In Proceedings of the Annual Meeting of the Latin American & Caribbean Aquaculture Societies, Bogota, Colombia, 23–26 October 2018.
44. Werlinger, C.; Mansilla, A.; Villarroel, A.; Palacios, M. Effects of photon flux density and agricultural fertilizers on the development of *Sarcothalia crispata* tetraspores (Rhodophyta, Gigartinales) from the Strait of Magellan, Chile. *J. Appl. Phycol.* **2008**, *20*, 757–765. [CrossRef]
45. Buschmann, A.H.; Varela, D.; Cifuentes, M.; Carmen Hernández-González, M.C.; Henríquez, L.; Westermeier, R.; Correa, J.A. Experimental indoor cultivation of the carrageenophytic red alga *Gigartina skottsbergii*. *Aquaculture* **2004**, *241*, 357–370. [CrossRef]
46. Mansilla, A.; Palacios, M.; Navarro, N.P.; Avila, M. Growth and survival performance of the gametophyte of *Gigartina skottsbergii* (Rhodophyta, Gigartinales) under defined nutrient conditions in laboratory culture. In Proceedings of the 19th International Seaweed Symposium, Kobe, Japan, 26–31 March 2007.
47. Arbaiza, S. Viabilidad Reproductiva para el Cultivo de *Chondracanthus chamissoi* Proveniente de Tres Poblaciones Del Litoral Peruano. Master's Thesis, Universidad Nacional Agraria La Molina, Lima, Peru, 2016.
48. Fernández-Linares, L.; Durán-Páramo, E.; Guerrero-Barajas, C. A scale-up evaluation of a semicontinuous culture of *Scenedesmus* sp. in a raceway under greenhouse conditions using a commercial fertilizer as culture medium. *Biofuels* **2021**, *12*, 1291–1299. [CrossRef]
49. Roleda, M.Y.; Hurd, C.L. Seaweed nutrient physiology: Application of concepts to aquaculture and bioremediation. *Phycologia* **2019**, *58*, 552–562. [CrossRef]
50. Harrison, P.J.; Hurd, C.L. Nutrient physiology of seaweeds: Application of concepts to aquaculture. *Cah. Biol. Mar.* **2001**, *42*, 71–82.
51. Demetropoulos, C.L.; Langdon, C.J. Enhanced production of Pacific dulse (*Palmaria mollis*) for co-culture with abalone in a land-based system: Effects of seawater exchange, pH, and inorganic carbon concentration. *Aquaculture* **2004**, *235*, 457–470. [CrossRef]
52. Matos, J.; Costa, S.; Rodrigues, A.; Pereira, R.; Sousa-Pinto, I. Experimental integrated aquaculture of fish and red seaweeds in Northern Portugal. *Aquaculture* **2006**, *252*, 31–42. [CrossRef]
53. Abreu, M.H.; Pereira, R.; Yarish, C.; Buschmann, A.H.; Sousa-Pinto, I. IMTA with *Gracilaria vermiculophylla*: Productivity and nutrient removal performance of the seaweed in a land-based pilot scale system. *Aquaculture* **2011**, *312*, 77–87. [CrossRef]
54. Grote, B. Recent developments in aquaculture of *Palmaria palmata* (Linnaeus) (Weber & Mohr 1805): Cultivation and uses. *Rev. Aquac.* **2019**, *11*, 25–41.
55. Nagler, P.L.; Glenn, E.P.; Nelson, S.G.; Napolean, S. Effects of fertilization treatment and stocking density on the growth and production of the economic seaweed *Gracilaria parvispora* (Rhodophyta) in cage culture at Molokai, Hawaii. *Aquaculture* **2003**, *219*, 379–391. [CrossRef]
56. Msuya, F. The effect of stocking density on the performance of the seaweed *Ulva reticulata* as a biofilter in earthen pond channels, Zanzibar, Tanzania. *West. Indian Ocean J. Mar. Sci.* **2008**, *6*, 65–72. [CrossRef]
57. Msuya, F. Effects of stocking density and additional nutrients on growth of the commercially farmed seaweeds *Eucheuma denticulatum* and *Kappaphycus alvarezii* in Zanzibar, Tanzania. *TaJONAS* **2013**, *4*, 605–612.
58. Corey, P.; Kim, J.K.; Duston, J.; Garbary, D.J. Growth and nutrient uptake by *Palmaria palmata* integrated with Atlantic halibut in a land-based aquaculture system. *Algae* **2014**, *29*, 35. [CrossRef]
59. Alveal, K. Estrategias Reproductivas de Rhodophyta y Sus Nexos Con la Biodiversidad. In *Sustentabilidad de la Biodiversidad. Un Problema Actual: Bases Científico-Técnicas. Teorizaciones y Proyecciones*; Alveal, K., Antezana, T., Eds.; Universidad de Concepción: Concepción, Chile, 2001; pp. 367–388.
60. Bulboa, C.R.; Macchiavello, J.E.; Oliveira, E.C.; Fonck, E. First attempt to cultivate the carrageenan-producing seaweed *Chondracanthus chamissoi* (C. Agardh) Kützing (Rhodophyta; Gigartinales) in Northern Chile. *Aquac. Res.* **2005**, *36*, 1069–1074. [CrossRef]
61. Fonck, E.; Martínez, R.; Vásquez, J.; Bulboa, C. Factors that affect the re-attachment of *Chondracanthus chamissoi* (Rhodophyta, Gigartinales) thalli. *J. Appl. Phycol.* **2007**, *20*, 311–314. [CrossRef]

62. Sáez, F.; Macchiavello, J.; Fonck, E.; Bulboa, C. The role of the secondary attachment disc in the vegetative propagation of *Chondracanthus chamissoi* (Gigartinales, Rhodophyta). *Aquat. Bot.* **2008**, *89*, 63–65. [CrossRef]
63. Rodríguez, C.Y.; Otaíza, R. Factors affecting morphological transformation and secondary attachment of apexes of *Chondracanthus chamissoi* (Rhodophyta, Gigartinales). *J. Appl. Phycol.* **2018**, *30*, 1157–1166. [CrossRef]
64. Maggs, C.A.; Callow, M.E. *Algal spores. Encyclopedia of Life Sciences*; Nature Publishing Group: London, UK, 2002; pp. 1–6.
65. Bischof, K.; Rautenberger, R. Seaweed responses to environmental stress: Reactive oxygen and antioxidative strategies. In *Seaweed Biology: Novel Insights into Ecophysiology, Ecology and Utilization*; Wiencke, C., Bischof, K., Eds.; Springer: Berlin, Germany, 2012; pp. 109–132.
66. Rydgren, K.; Økland, R.H. Short-term Costs of Sexual Reproduction in the Clonal Moss Hylocomium splendens. *Bryologist* **2003**, *106*, 212–220. [CrossRef]
67. Halling, C.; Aroca, G.; Cifuentes, M.; Buschmann, A.H.; Troell, M. Comparison of spore inoculated and vegetative propagated cultivation methods of Gracilaria chilensis in an integrated seaweed and fish cage culture. *Aquac. Int.* **2005**, *13*, 409–422. [CrossRef]
68. Guillemin, M.L.; Valenzuela, P.; Gaitán-Espitia, J.D.; Destombe, C. Evidence of reproductive cost in the triphasic life history of the red alga *Gracilaria chilensis* (Gracilariales, Rhodophyta). *J. Appl. Phycol.* **2014**, *26*, 569–575. [CrossRef]
69. Deza, K.; Gil-Kodaka, P.; Fernández, E.; Mendo, J. Efecto del tamaño de corte sobre la tasa de crecimiento y cobertura de la *macroalga Chondracanthus chamissoi* "yuyo" de la zona submareal de Playa Mendieta, Paracas, Pisco. In Proceedings of the Memorias I Jornada Científica Reserva Nacional Paracas, Lima, Peru, 28–31 March 2001.
70. Gil-Kodaka, P.; Mendo, J.; Fernández, E. Diversidad de macroalgas del submareal en la Reserva Nacional de Paracas y notas sobre su uso potencial. In Proceedings of the Memorias I Jornada Científica Reserva Nacional Paracas, Lima, Peru, 28–31 March 2001.
71. Sullivan, G.M.; Feinn, R. Using effect size-or why the *P* value is not enough. *J. Grad. Med. Educ.* **2012**, *4*, 279–282. [CrossRef]
72. Lakens, D. Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVA. *Front. Psychol.* **2013**, *4*, 863. [CrossRef] [PubMed]
73. Fox, J.; Weisberg, S. *An R Companion to Applied Regression*, 3rd ed.; Sage: Los Angeles, CA, USA, 2019; pp. 1–608.
74. Hothorn, T.; Bretz, F.; Westfall, P. Simultaneous inference in general parametric models. *Biom. J.* **2008**, *50*, 346–363. [CrossRef] [PubMed]
75. Zeileis, A.; Kleiber, C.; Jackman, S. Regression Models for Count Data in R. *J. Stat. Softw.* **2008**, *27*, 1–25. [CrossRef]
76. Jackman, S. pscl: Classes and Methods for R Developed in the Political Science Computational Laboratory. United States Studies Centre, University of Sydney, Sydney, New South Wales, Australia. R Package Version 1.5.5. 2020. Available online: https://github.com/atahk/pscl/ (accessed on 12 February 2023).
77. Venables, W.N.; Ripley, B.D. *Modern Applied Statistics with S*, 4th ed.; Springer: New York, NY, USA, 2002; pp. 1–510.
78. Sellke, T.; Bayarri, M.J.; Berger, J.O. Calibration of *p* values for testing precise null hypotheses. *Am. Stat.* **2001**, *55*, 62–71. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.