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# Settlement and growth of *Chondracanthus chamissoi* (Rhodophyta, Florideophyceae) carpospores on three types of substrate under semi-controlled laboratory conditions

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**Abstract:** *Chondracanthus chamissoi* (C. Agardh) Kützing (1843) is a red seaweed extracted along the Peruvian coast, due to its commercial importance for human consumption and the extraction of carrageenan. Despite this, in Peru there is little research aimed at the development of its cultivation. Therefore, the present study evaluated the settlement of carpospores and the growth of the attachment disc of *C. chamissoi* on three types of artificial substrate: nylon, raffia and polypropylene ropes under laboratory conditions, making it the first comparative study about substrate effects on this species' development. The experiment lasted 28 days. The highest settlement was obtained on the raffia substrate (316 ± 74.48 carpospores.cm$^{-2}$), showing significant differences ($p < 0.05$) respect to the nylon substrate (185 ± 26.93 carpospores.cm$^{-2}$) and the polypropylene substrate (170 ± 37.03 carpospores.cm$^{-2}$). Likewise, the diameter of the attachment discs was significantly greater ($p < 0.05$) for nylon (40.04 ± 1.17 µm) and raffia (38.65 ± 0.90 µm) ropes compared to polypropylene ropes (32.97 ± 1.39 µm).

**Key words:** Macroalgae, culture, seed production, artificial substrates, roughness.

**Asentamiento y crecimiento de carposporas de *Chondracanthus chamissoi* (Rhodophyta, Florideophyceae) sobre tres tipos de sustrato en condiciones semi-controladas de laboratorio. Resumen:** *Chondracanthus chamissoi* (C. Agardh) Kützing (1843) es una macroalga roja extraída a lo largo de la costa peruana, debido a su importancia comercial para consumo humano y la extracción de carragenano. A pesar de ello, en el Perú son escasas las investigaciones orientadas al desarrollo de su cultivo. Por lo tanto, el presente estudio evaluó el asentamiento de carpósporas y crecimiento del disco de adhesión de *C. chamissoi* en tres tipos de sustrato artificial: cuerdas de nylon, rafia y polipropileno en condiciones de laboratorio. El experimento tuvo una duración de 28 días. El mayor asentamiento se obtuvo en sustrato rafia (316 ± 74,48 carposporas.cm$^{-2}$) mostrando diferencias significativas ($p < 0.05$) respecto al sustrato nylon (185 ± 26,93 carposporas.cm$^{-2}$) y al sustrato polipropileno (170 ± 37,03 carposporas.cm$^{-2}$). Así mismo, el diámetro de los discos de adhesión fue significativamente mayor ($p < 0,05$) para las cuerdas de nylon (40,04 ± 1,17 µm) y rafia (38,65 ± 0,90 µm) en comparación con las cuerdas de polipropileno (32,97 ± 1,39 µm).

**Palabras clave:** macroalgas, cultivo, producción de semillas, sustratos artificiales, rugosidad.

## Introduction

*Chondracanthus chamissoi* (C. Agardh) Kützing (1843), is a red seaweed from to the Gigartinaceae family and endemic to the temperate coasts of Southeast Pacific Ocean. It is distributed from Paita, Peru (5º) to Ancud, Chile (42 ° S), however, it has also been also reported in Japan and Korea (Acleto 1986, Yang *et al.* 2015). In Peru, it is currently consumed in the national gastronomy in a fresh state, for the preparation of marine dishes, and has been part of the diet since pre-Inca times (Carbajal *et al.* 2005). Likewise, it is one of the most abundant seaweed resources on the Peruvian coast, which sustains the economy of various groups of artisanal fishermen who dedicate themselves to the manual extraction at the intertidal zone or by autonomous diving at the subtidal zone (Acleto 1986, Carbajal *et al.* 2005). Additionally, this marine resource is constantly extracted for κ-carrageenan and λ-carrageenan type phycocolloids obtention, which are used in the food industry as thickeners and gelling agents (Hoffmann & Santelices 1997, Salas *et al.* 2008). For its high extractive pressure, there is a risk of reducing its abundance in Peruvian natural beds (Flores *et al.* 2015).

The cultivation of *C. chamissoi* is mainly done by using two techniques: vegetative cultivation, which consists in the regeneration of previously fragmented fronds, whose advantage is to obtain genetic and phenotypically similar organisms from an original parental thallus (Sáez *et al*. 2008, Bulboa *et al*. 2013); and spore cultivation, which generates individuals with different genotypes providing high yields due to the large number of spores that are produced from a low biomass of reproductive individuals (Bulboa *et al*. 2019). Some studies related to spore cultivation settled on ropes have been carried out in Peru (Castañeda *et al*. 2018, Arbaiza *et al*. 2019) and Chile (Alveal *et al*. 1999, Bulboa & Macchiavello 2001, Macchiavello *et al*. 2003, Bulboa & Macchiavello 2006, Ávila *et al*. 2011). Likewise, some problems related to survival, spore release, low growth rates and epibionts have been reported in cultivation from spores (Bulboa *et al*. 2005, Bulboa & Macchiavello 2006, Bulboa *et al*. 2007). Despite this, obtaining *C. chamissoi* spores in controlled or semi-controlled environments represents a great advantage due to the ease of handling and the high production that could potentially be obtained (Macaya 2001, Bulboa *et al*. 2013).

During the cultivation process, factors such as temperature, photoperiod, nutrient concentration and pH are taken into account to ensure its success (Agrawal 2009). Also, the surface structure of the substrate is a relevant since it is the physical environment where the inoculums are developed (Navarro 2000). Characteristics such as texture, hardness, roughness and heterogeneity of the substrate, are decisive for the development of spores on them (Castañeda *et al*. 2018, Arbaiza *et al*. 2019). In addition, the ropes should be considered as efficient and easy-to-handle substrates for a cultivation process with productive purposes (Foster 1980). The use of ropes have been recorded for the cultivation under controlled conditions of spores of *Sarcothalia crispata* (Ávila *et al*. 1996, Romo *et al*. 2001, Hughes *et al*. 2018), *Chondracanthus chamissoi* (Bulboa & Macchiavello 2006, Bulboa *et al*. 2010, Ávila *et al*. 2011, Arbaiza *et al*. 2019), *Gracillaria chilensis* (Alveal *et al*. 1997), *Gracillaria gigas* (Irwani 2013) and *Gracilaria gracilis* (Hughes *et al*. 2014), where different related variables have been considered to growth and settlement in the different substrates of nylon, raffia and polypropylene. Therefore, the choice of a substrate with suitable surface patterns will optimize the recruitment, settlement and viability of the spores (Fletcher & Callow 1992, Buschmann *et al*. 2008). The objective of the present study was to determine the settlement and growth of *C. chamissoi* carpospores using three different types of ropes commonly used in Peru (nylon, raffia and polypropylene) in semi-controlled laboratory conditions. This is the first comparative study between different types of substrates for the cultivation of *C. chamissoi* caropospores in Peru

## Materials and methods

*Design, preparation and treatment of artificial substrates:* Previously to the development of the experimental phase, the settlement systems were made using PVC tubes (0.5 inches) to build frames (33 cm long and 23 cm wide) on which the different ropes that would be used as artificial substrates were wound. The ropes used as artificial substrate were made of three different types: nylon, raffia and polypropylene (Table I; Fig. 1), and were previously washed, rinsed and maintained in culture tanks with

**Table I.** Observable characteristics for the three types of ropes used at the experimental phase.

| **Nylon ropes** | **Raffia ropes** | **Polypropylene ropes** |
|---|---|---|
| ***Diameter**:* 5.0 mm<br>***Material:*** artificial polymer with a very dense white elastic imbricated braiding<br>***Texture:*** Homogeneous rough, miscible with water | ***Diameter:*** 7.0 mm<br>***Material:*** extruded synthetic fibers with a flexible twisted helical braid of three ropes<br>***Texture:*** Heterogeneous surface, immiscible with water | ***Diameter:*** 7.0 mm<br>***Material:*** three twisted braided cords, where each cord is made up of smooth multifilament.<br>***Texture:*** smooth, hard to the touch and miscible with water |


**Figure 1**. Detail of ropes used at the experimental phase: nylon (A), raffia (B) and polypropylene (C).

50 µm filtered seawater for four weeks in order to induce the formation of bacterial biofilms to favor the recruitment and settlement of carpospores (Maggs & Callow 2002, Agrawal 2009).

Three frames were made for each type of ropes as a repetition. In each frame, 12 ropes of 2.5 cm length were included as control samples and randomly distributed in each settlement system. It should be noted that the samples were of the same type of rope that was being used in the frame.

Subsequently, the frames were randomly submerged in three 40-L capacity culture tanks with 0.22 µm-filtered seawater and constant aeration. Water changes were made weekly and the commercial nutrient Bayfolan® was added in a concentration of 0.1 ml $L^{-1}$ as culture medium. The culture conditions were kept constant during the four weeks of evaluation: photoperiod 12:12 (Light: dark), temperature (16 ± 1.38 ºC), salinity (38 ± 0.81 psu), pH (8 ± 0.17), dissolved oxygen (7 ± 1 mg $L^{-1}$) and the irradiance was maintained at 4 µmol foton.$m^{-2}s^{-1}$ as a culture strategy to avoid epiphytes during the experimental process. It should be noted that, during the first week of evaluation, aeration was not placed in the system, in order to avoid the dispersion of spores towards unwanted settlement areas. The experimental procedures were carried out in the Laboratorio de Investigación en Cultivos Marinos (LICMA) located in the district of San Andrés, Pisco (Peru).

*Collection and treatment of C. chamissoi:* The collection was made by members of the Cooperativa de Trabajadores Pesqueros Artesanales Algas Marinas (COTRAPLMAR), who by autonomous diving collected cystocarpic individuals of *C. chamissoi* from Playa Mendieta located in the Paracas National Reserve (14º 3 '23.4' 'S, 76º 15 '39.6' 'O). Subsequently, they were hermetically transported at 10 ºC to the LICMA facilities. In the laboratory, the fronds went through a cleaning and disinfection process that consisted of washing it with a sodium hypochlorite solution (1%) for 10 seconds, to eliminate epiphytes (e.g. other algae spores not target in the study, diatoms and others epiphytes). Subsequently, the fronds of *C. chamissoi* were rinsed and washed with abundant sea water, rubbing gently, to avoid breaking them and eliminating the rest of impurities.

*Induction of sporulation:* Carposporophytic fronds were induced to sporulation, using desiccation stress (sun exposure) between 25 to 30 minutes considering a consistency of partial dehydration (sticky texture). After this time, fronds were separated into three groups of 500 g each and distributed entirely on fishing mesh (25.4 mm opening) suspended on top of the culture tanks to promote spore release throughout the area of the settling system for 48 hours. Subsequently, the fronds that released its spores were removed and three control samples of each type of string were taken to calculate the initial amount of sedimented spores, where a value of 400 - 450 $cm^{-2}$ spores was obtained from each experimental unit.

*Settlement on ropes:* Settlement was monitored by taking photographs of spores attached throughout the control sample of each treatment using a stereoscope (Leica EZ4E) on a weekly basis. To determine the number of spores settled per area at the end of the experiment, the formula for the lateral area of a cylinder (AL) was applied and it was divided by two, because the accounting was only carried out in the area exposed to spore rain:

$$AL = \frac{2 * \pi * r * h}{2}$$

where $r$ is the radius of the evaluated chord and $h$ is the height (2.5 cm).

*Attachment disc growth:* Random measurements of *C. chamissoi* carpospores were made, obtaining an initial size of 10.89 ± 0.96 µm (n = 90 carpospores). The diameter of the attachment discs, of each type of rope used during the study, was measured on the last day of evaluation (n=60), having a total of three ropes measured in triplicate. Measurements were made using Leica model: EZ4E stereoscope software. With the data obtained for each substrate, the specific growth rate (TC) was determined with the following formula proposed by Bulboa *et al*. (2010):

$$TC(\%) = \left\{ \left[ \left( \frac{Lf}{Li} \right)^{t^{-1}} \right] - 1 \right\} * 100$$

where $TC$ is the specific growth rate, $Lf$ is the final length, $Li$ is the initial length and $t$ is the period of time expressed in days of culture.

*Statistical analysis:* The settlement of carpospores was analyzed with non-parametric tests since they were significant for the Shapiro-Wilk and Bartlett tests ($p$ <0.05). Therefore, the non-parametric Kruskall-Wallis test was used, in order to make comparisons of each variable between nylon, raffia and polypropylene ropes. For the evaluation of the growth of the attachment discs, normal distribution and homogeneity of variances were determined, for which the parametric ANOVA test was used and multiple comparisons were analyzed by Tukey's test. The R Studio program was used for data analysis.

## Results

*Settlement on ropes:* After 04 weeks, the settlement of *C. chamissoi* carpospores was evidenced for the three substrates evaluated (Fig. 2). The highest settlement was obtained in the raffia ropes (316 ± 74.48 carpospores.$cm^{-2}$) showing significant differences compared to nylon ropes (185 ± 26.93 carpospores.$cm^{-2}$) and polypropylene ropes (170 ± 37.03 carpospores $cm^{-2}$) (Table II). Also, a decrease in the density of carpospore settlement was observed (Fig. 2).

*Attachment disc growth:* The diameter of the attachment discs was significantly bigger ($p$ < 0.05) for nylon (40.04 ± 1.17 µm) and raffia ropes (38.65 ± 0.90 µm) compared to polypropylene ropes (32.97 ± 1.39 µm) (Table II). Likewise, *TC* showed significant differences for the evaluated treatments ($p$ < 0.05), having growth rates of 4.78% $d^{-1}$ for nylon, 4.63% $d^{-1}$ for raffia and 4.02 % $d^{-1}$ for polypropylene ropes (Table III).

## Discussion

*Settlement on ropes:* In the three evaluated substrates, different settlement densities were evidenced (Table II), showing that there is a clear affinity for the raffia substrate, followed by nylon and polypropylene ropes (Fig. 2). Likewise, the differences in the roughness and heterogeneity patterns of the contact surface have been observed and considered (Fig. 1), characteristics that are appropriate to facilitate correct adhesion and subsequent growth of the attachment disc (Lobban & Harrison 1994, Fletcher & Callow 1992).

Polypropylene ropes obtained lower densities due to the rough surface characteristics of the substrate, which did not allow the fixation and successful development of the spores, since twisted braided ropes present multifilaments with a smooth surface. This characteristic facilitates the sliding of the spore towards non-target settlement areas and could condition its survival (Fletcher & Callow 1992, Navarro 2000). Despite this, Arbaiza *et al* (2019), carried out a cultivation experiment under laboratory conditions using polypropylene ropes, where after 120 days of experiment they obtained 49.0 $cm^2$ seedlings of *C. chamissoi*. In *Sarcothalia crispata*, differences were observed in spore recruitment both on natural substrates and on 2 mm and 5 mm polypropylene ropes, finding higher survival rates (around 80%) and carpospore densities (200 individuals $m^2$) in shells clams and pebbles, which present different degrees of visible roughness (Ávila et al. 1996). Studies carried out by Foster (1980) and Fletcher & Callow (1992) mention that the soft textures of artificial substrates promote the sliding of algae spores, preventing and reducing settlement on substrates with this type of characteristics, so in this study, it was determined that the polypropylene substrate was the least

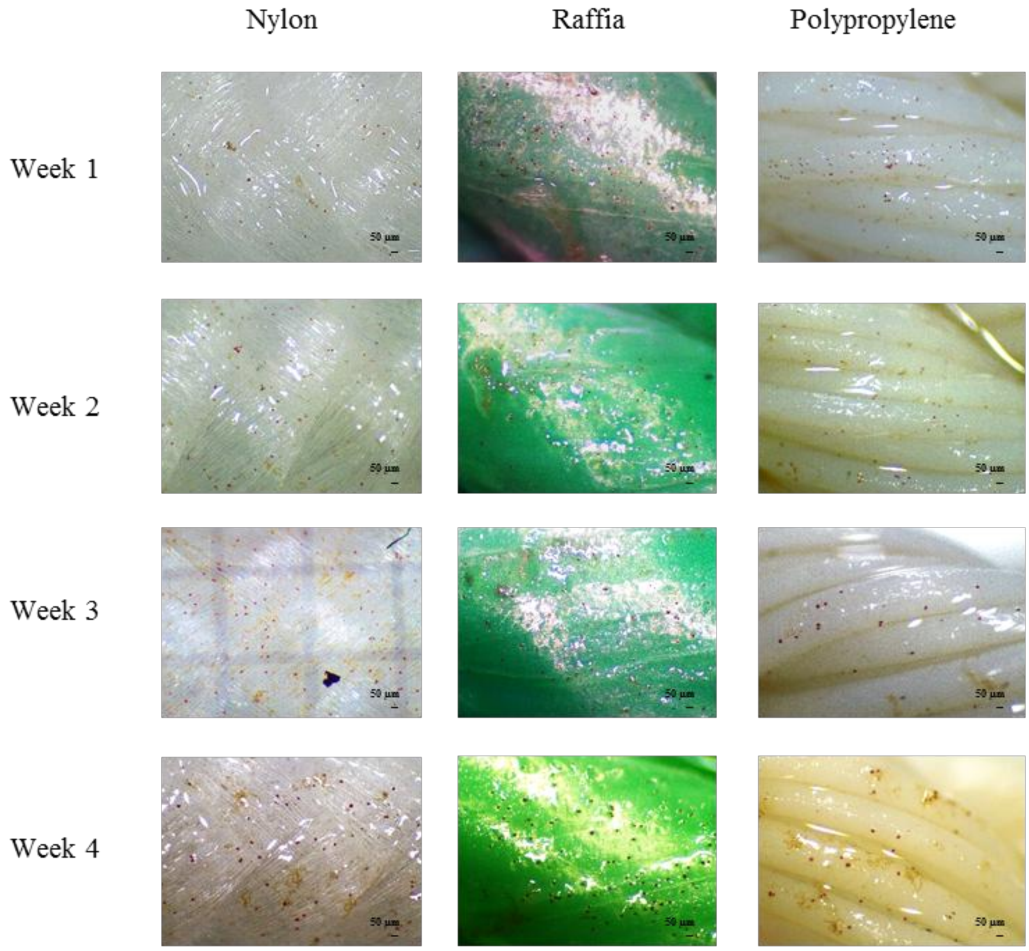


**Figure 2.** Weekly evaluation of the settled carpospore density of *Chondracanthus chamissoi* for four weeks on nylon, raffia and polypropylene ropes under semi-controlled conditions. Magnification: 35X.

**Table II.** Density of settled carpospores of *Chondracanthus chamissoi* per $cm^2$ for nylon, raffia and polypropylene substrates after 28 days of evaluation. Lowercase letters indicate significant differences ($p < 0.05$).

| Treatment | Mean ± 95% CI | Max | Min |
|---|---|---|---|
| Nylon | 185 ± 29.63[b] | 223 | 105 |
| Raffia | 316 ± 74.48[a] | 524 | 211 |
| Polypropylene | 170 ± 37.03[b] | 244 | 97 |

a, b Different letters indicate significant differences determined by the Kruskal-Wallis test ($p < 0.05$).

suitable for the settlement of *C. chamissoi* because it presented a smooth surface pattern.

Regarding the raffia and nylon ropes, Irwani (2013), recorded a greater settlement and development of *Gracilaria gigas* spores in raffia ropes with 80 thalli $cm^2$, followed by nylon ropes with around 40 thalli $cm^2$ during two months of evaluation, due to the heterogeneous surface of the raffia rope. This trend and surface characteristic of the rope was also observed in the present investigation, obtaining higher settlement densities in raffia ropes (316 ± 74.48 carpospores $cm^2$) compared to nylon ropes (185 ± 26.93 carpospores $cm^2$). Hughes *et al*. (2014), found a higher density of carpospores settled in shells (around 40 spores $mm^2$) compared to pebbles (around 5 spores $mm^2$) where lower densities of *Gracilaria gracilis* carpospores were observed. Hughes *et al*. (2018), found greater settlement of *Sarcothalia crispata* carpospores in the natural substrates of gravel (79.11 spores $cm^2$) and bivalve shells (61.13 spores $cm^2$) and obtained lower settlements with the 3 mm nylon ropes towards the end of the experimental process with 14.45 spores $cm^2$, however in the fourth week of evaluation the preference for shell substrates followed by nylon ropes was evidenced, as well as the decrease in $cm^2$ carpospores as the weeks passed evaluation.

It is worth mentioning that natural substrates, such as the rocky areas of the intertidal and subtidal areas where *C. chamissoi* lives and develops (Acleto 1986), present surfaces with imperfections and roughness that allow the adequate settlement, attachment, and development of the attachment disc of the spore and that must be considered when selecting a certain substrate (Romo & Alveal 1995). However, despite the fact that better results are obtained using natural substrates (pebbles, gravel and bivalve shells) compared to artificial substrates (ropes), as mentioned above, it should be emphasized that cultivation with synthetic fiber-based ropes benefit management when carrying out a larger-scale cultivation and these can also be manufactured with physical roughness patterns suitable to achieve the development of spore settlement and attachment (Macaya 2001, Foster 1980). Likewise, Doty & Fisher (1978) mention that ropes derived from plastic, such as nylon and raffia, are optimal for the recruitment of *Gracilaria* sp. spores. However, raffia ropes obtained the most satisfactory and adequate results of settlement due to the nature of the substrate and the surface properties it presents (Fig. 2).

In the present work, we can find the same pattern as Hughes et al. (2018) who evidenced the decrease in settlement as the weeks of experimentation passed. Likewise, Romeo et al. (2001), mention that the decrease in settlement over time is more related to the presence of spores that have not yet completed their maturation process, rather than to the effect of abiotic variables, such as temperature and photoperiod. On the other hand, the use of lower irradiance (4 µmol photon.$m^2s^{-1}$) in our study had a positive influence on the development of *C. chamissoi* carpospores since no epiphytes were observed during the four weeks of evaluation. The irradiance value commonly used (60 - 70 µmol photon.$m^2s^{-1}$) for this species' cultivation could facilitate the presence of epiphytes, especially, zooids of *Ulva* sp. (Bulboa et al. 2007)

Considering the characteristics of the substrate, Maggs & Callow (2002), mention that the spores have greater compatibility with substrates with water-inmiscible surfaces, because this characteristic favors the formation of the bacterial biofilm capable of modifying the surface adhesion properties of the spores to the substrate, allowing a better settling process. This hydrophobic surface has been observed as a property of raffia ropes, in addition to the rough surface characteristics it presents, for which better results are attributed to it for the settlement of *C. chamissoi* carpospores.

*Attachment disc growth:* The measurement and specific growth rates of the attachment discs showed higher values, both for the nylon ropes and for the raffia ropes, showing no significant differences between them, compared to the polypropylene ropes ($p$ > 0.05) (Table III). Hughes et al. (2014), evaluated the growth rate of attachment discs formed by *Gracilaria gracilis* carpospores in two types of natural substrates, obtaining better growth rates of the attachment discs in pebble substrates (7.59% $day^{-1}$) compared to shell substrates (5.67% $day^{-1}$) indicating that the heterogeneous surface provided by pebbles facilitates the growth of the *G. gracilis* attachment disc. In our study it happens in a similar way, polypropylene ropes presented the lowest growth rates of the attachment disc (4.02% $day^{-1}$) being the smoothest rope, compared to nylon ropes (4.78% $day^{-1}$) and raffia (4.63% $day^{-1}$) (Table III).

On the other hand, the diameter values of the attachment disc, for nylon (40.04 ± 1.17 µm) and raffia (38.65 ± 0.90 µm) substrates, showed significant differences compared to the polypropylene substrate (32.97 ± 1.39 µm). (Table III). Compared to Avila et al. (2011), we obtained smaller diameters in the same amount of time (4 weeks= 280 um); as well as in other species such as: *Gracilaria dura* with 1200 µm (Mantri et al. 2009), *G. gracilis* with 500 µm (Hughes et al. 2014) and *S. crispata* with 125.44 ± 7.03 µm (Hughes et al. 2018). Probably this difference could be attributed to the low irradiance value of 4 µmol photon $m^2s^{-1}$.

Bulboa et al. (2010), using an irradiance of 60 µmol photon $m^2s^{-1}$, obtained growth rates between 5.3 ± 0.4% and 9.3 ± 0.2% $d^{-1}$ of the attachment discs of *C. chamissoi* from two locations; values that exceed the growth rates compared to our results (between 4.02 - 4.78% $d^{-1}$). In our case, the irradiance value was well below of that used for the development of *C. chamissoi* carpospores (60 - 70 µmol photon $m^2s^{-1}$) (Gonzales & Meneses 1996, Bulboa et al. 2010). Although the use of a low irradiance is not limiting for the settlement of *C. chamissoi* carpospores, in terms of the growth of the attachment discs, it turns out to be a determining variable (Bulboa & Macchiavello 2001, Agrawal 2009).

Finally, our results show that the raffia ropes have adequate superficial physical characteristics, which favor a higher settlement and growth rate of the attachment discs of *C. chamissoi*. However, the results indicate that although this study has shown that raffia is the most suitable artificial substrate to start a culture of *C. chamissoi* carpospores under

**Table III.** Diameter (µm) and specific growth rate (TC, % $d^{-1}$) of attachment discs (n=60) from *Chondracanthus Chamissoi* on different artificial substrates after 28 days of evaluation. Lowercase letters indicate significant differences ($p < 0.05$).

| Substrates | Diameter (µm) ± 95% CI | TC (% $d^{-1}$) |
|---|---|---|
| Nylon | 40.04 ± 1.17[a] | 4.78[a] |
| Raffia | 38.65 ± 0.90[a] | 4.63[a] |
| Polypropylene | 32.97 ± 1.39[b] | 4.02[b] |

[a, b] Different letters indicate significant differences determined by the Kruskal-Wallis test ($p < 0.05$).

semi-controlled laboratory conditions, the other two substrates show characteristics that also serve for adhesion and growth of the attachment discs and it is expected that these laboratory results provide referential information for future experiments incorporating new variables such as irradiance.

## Ethical statement

The present investigation did not involve regulated animals and did not require approval by an Ethical Committee.

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