

Short Communication

Experimental transplantation of *Durvillaea incurvata* in southern Chile: implication for its restocking

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ABSTRACT. *Durvillaea incurvata* is a brown macroalgae of ecological, social, and economic importance subjected to a drastic increase in harvesting pressure over the last decade in Chile. In this study, we performed an experimental transplantation of juveniles of *D. incurvata*, assessing the potential use as a restocking technique. Different types of restocking devices and attaching substrates were constructed. Differences in the *D. incurvata* re-attachment probability among the substrates and devices were not observed, and a high percentage of juvenile mortality was observed. However, reattached juveniles increased their length, holdfast diameter, and weight. The experiment revealed that juveniles of *D. incurvata* have a good capacity for re-attachment and growth on artificial substrates after transplantation. This technique could be used for the species stock enhancement, although further studies are needed to optimize the process and increase the juvenile's re-attachment.

Keywords: *Durvillaea incurvata*; growth rate; restocking-devices; transplant; re-attachment; stock enhancement

Brown macroalgae are important primary producers and ecosystem engineers playing a central role in the ecology of coastal temperate habitats (Teagle et al. 2017), providing ecosystem services along 25% of the world's coastlines (Filbee-Dexter & Wernberg 2018). However, global stressors, such as climate change and biotic homogenization, along with local stressors, such as habitat modification, eutrophication, overgrazing, trampling, and harvesting, can lead to changes in their population density (Mineur et al. 2015). As ecosystem engineers, the deterioration of macroalgae populations also affects the local biodiversity they sustain (Mineur et al. 2015, Krumhansl et al. 2016). Nowadays, there are restocking and restoration methods that are poten-

tially useful to recover and conserve the macroalgae-threatened populations (Carney et al. 2005, Gianni et al. 2013, Westermeier et al. 2016, Oyarzo-Miranda et al. 2023).

The restoration of kelp beds is a complex process because these inhabited zones are exposed to wave action and tidal forces that can result in high mortality of the settling embryos and juveniles (e.g. Santelices et al. 1980). Kelps compensate by producing abundant spores with high recruitment levels, but as they increase their size, the herbivores can remove individuals from the rocks (Santelices et al. 1980, Westermeier et al. 1994). Moreover, the desiccation can reduce the individual's survival, particularly of those settled in the

middle or upper intertidal zones where higher temperatures can be recorded (e.g. Hay 1979). The action of waves and high temperature can exert important effects on survival during the restocking process because the spores/individuals generated in a hatchery have to acclimate physiologically to the new substrates and environmental conditions (e.g. Vásquez & Tala 1995, Correa et al. 2006, Westermeier et al. 2016). The design and construction of restocking devices are also fundamental to allow efficient individual holdfast anchoring on the rocks (Correa et al. 2006, Oyarzo-Miranda et al. 2023). The restocking-device improvement must consider the human effort to their fixation to the rock, the device economic cost, and the device efficiency to support the biomass increase over time.

In Chile, *Durvillaea antarctica* (Cham.) Hariot and *D. incurvata* (Suhr) Macaya are important social, economic, and ecological brown macroalgae with non-overlapping distributions along the Pacific Ocean coast. Fraser et al. (2020) reviewed the *Durvillaea* genus, concluding that *D. antarctica* is distributed from Cape Horn to Isla Betecoi (43°S), and a new species, *D. incurvata* is distributed from Isla Betecoi to 30°S. Hence, previous studies performed with *D. antarctica* between 43 and 30°S should be considered *D. incurvata*. This conspicuous brown macroalgae (Santelices et al. 1980) has experienced strong harvesting pressure over the years. In 2007, 4274 wet tons of *Durvillaea* were harvested in Chile, increasing to 11,602 wet tons in 2022 (SERNAPESCA 2023). Historically, *D. incurvata* has been exploited exclusively from coastal populations threatening the species. In 2015, the first extractive ban for the species (Dec. Ex. N°759-2015) was ordered in central Chile (O'Higgins Region, ca. 34°S, 71°W). Since then, an increasing number of areas have been subject to bans orders due to the risk to the population's sustainability. Despite the ecological and cultural relevance of the *Durvillaea* and the increasing population threat due to overharvesting, the species' capacity for restoration or restocking actions has not yet been assessed in Chile. This study aims to evaluate the use of the *D. incurvata* juvenile transplantation as a potential restocking technique for coastal populations.

The transplantation experiments of *D. incurvata* juveniles were performed in an intertidal population in Pucatrihue (40°32'57.40"S, 73°43'04.16"W), a coastal area exposed to the southeast Pacific Ocean, where *D. incurvata* beds are distributed from the high subtidal to the middle rocky intertidal (free-access zone). Two kinds of restocking devices were constructed with polyethylene or stainless steel: 1) a rectangular strip (50

cm in length and 10 cm wide) with four substrates-fixation bolts in the upper part, and 2) a truncated pyramid (15 cm in length, 10 cm wide, 8 cm top) with substrates-fixation bolts on each side (Figs. 1a-b). The strip device was fixed to rocks by two bolts and the pyramid device by one bolt (Figs. 1a-b). The bolts fixed on the restocking devices' surface were used to hold circular substrates (12 cm diameter) to which the transplanted juveniles were attached using stainless steel hooks (Figs. 1a-b). The substrates were constructed with stones collected in the rocky littoral, Technyl (polyamide), and polyethylene (Figs. 2a-b). The restocking devices were fixed to the rocks along the lower intertidal (eight rectangular strips and eight truncated pyramids). The experiment started with the juvenile collection in the intertidal of Pucatrihue on April 8, 2016; the holdfasts were cleaned with a scalpel and washed with filtered seawater to remove epibionts. Then, the maximum length (cm), holdfast diameter (cm), and the wet weight (g) were measured. The juveniles were fixed to the substrates on April 9, 2016, and the substrates were bolted to the restocking devices along with colored plastic identifications (Figs. 2a-b). Unfortunately, the tidal condition and wave action precluded the fixation of the same number of juveniles in all treatments. Hence, 61 juveniles were transplanted: 20 were fixed on stone, 21 on polyethylene, and 20 on Technyl substrates. Accordingly, 28 juveniles were maintained on the pyramid restocking device and 33 on the laminal strip device. After 30 days, the juveniles were examined to determine whether they were reattached to the substrates (Fig. 2c). The juvenile growth was evaluated only in those reattached after 30 days of the experimental initiation, and this time was considered the acclimation period. Later, the acclimation, we compared the maximum length growth rate (LGR) and the holdfast diameter growth rate (HGR) of juveniles reattached with juveniles from the natural population (n = 12), which acted as control (Fig. 2d) at the 50 and 140 days after the experiment initiation (Figs. 2e,i). The last morphological measures were made after 200 days of experimentation, then the individuals were removed, and the wet weight was recorded. The growth rates were estimated using the Yong et al. (2013) equation:

$$[(Lt/L0)^{1/t} - 1] \times 100 \%,$$

where Lt: final length (cm), L0: initial length (cm), t: time (d). For the transplantation experiment, the effects of the substrates (levels: stones, Technyl, and polyethylene) and restocking devices (levels: rectangular strip and pyramid) on the juvenile's probability (Pb) re-attachment were evaluated. A binomial residual

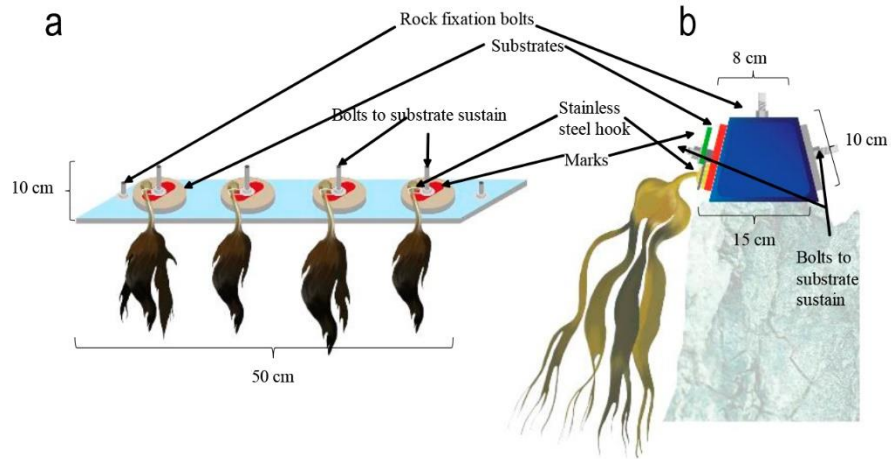


Figure 1. Illustration of the restocking devices constructed for the *Durvillaea incurvata* juveniles transplantation: a) strip and b) truncated pyramid types.



Figure 2. a-b) *Durvillaea incurvata* juveniles transplanted on the different substrates bolted in the strip restocking device; c) individuals fixed to the substrates and holdfast covering the steel hooks; d) individuals used as control and identified by purple plastic marks; e-f) individuals transplanted on truncated pyramid after ca. 140 days of experimentation; g-i) individuals, after ca. 140 days of experimentation, transplanted on a strip restocking device.

distribution model was fixed, and the ANOVA was performed in a generalized linear model (GLM) framework. In addition, the effects of the substrate (levels: stone, Technyl, polyethylene, and population control), time of experimentation (levels: 50, 140, and 200 days), and its interaction on the growth rates were evaluated using a Gaussian residual model and the ANOVA was performed using an LM framework. Finally, to evaluate the morphological changes of the individuals (maximum length, holdfast diameter, and wet weight) between the start and end of the experiment, ANOVAs were made using a Gaussian distribution of the residual model in an LM framework. When ANOVA with a Gaussian distribution model was used, the assumption of homoscedasticity and normality was tested with Levene's and Shapiro-Wilk's tests, respectively. A natural logarithm transformation was used when the normality assumption was not met. In each case, the null statistical hypotheses were rejected at a significance level (α) of 0.01. All analyses were made with R software (R Core Team 2020).

After 30 days of acclimation, 30 juveniles (49%) were reattached successfully to the substrates, and the other 31 juveniles (51%) suffered stipes damage or necrosis. In addition, after 140 and 200 days of experimentation, the 12 individuals used as control reduced their abundance to 6 (50%) and 4 (33%), respectively. It has been reported that *D. antarctica* sporelings cannot survive when exposed to hot and dry conditions due to physiological tolerance constriction to high temperature and desiccation (Hay 1979). The *D. incurvata* transplant experiment was performed in 2016. This year, southern Chile experienced the most severe drought and excessive solar irradiance observed in recent years, a climatic anomaly related to the concomitant strong El Niño and the positive polarity of the Southern Annular Mode (Garreaud 2018). Therefore, transplanted and control juveniles could have suffered the effects of desiccation, affecting re-attachment and survival.

The stripe restocking device showed a higher probability of re-attachment than the pyramid device (Pb: 0.515 ± 0.109 and 0.468 ± 0.108 , respectively). Meanwhile, the most successful re-attachment rate was observed on substrate stone (Pb: 0.597 ± 0.032), and the lowest on polyethylene (Pb: 0.379 ± 0.032); however significant effects on the juveniles re-attachment probability were not observed (GLM, $P > 0.01$). Despite no statistical differences, the economic and technical characteristics of the restocking devices and substrates are important to its selection. On the one hand, the stones used as substrates were recollected in

the nearby rocky littoral with no economic cost and minimal environmental disturbance. On the other hand, the strip device confers higher flexibility for fixation on uneven boulders, and the two bolts used for its fixation increase the resistance to wave action, which is key for the dragging forces experienced by juveniles in the natural environment. Therefore, we recommend the strip restocking device with stones for the *D. incurvata* juvenile transplantation.

The growth of *D. incurvata* was affected by the substrates and interacting factors, revealing contrasting growth responses. The control juveniles showed higher growth than those reattached to the substrates after 50 days of experimentation (LM, $P < 0.01$). The control juveniles had an LGR of $1.268 \pm 0.557\% \text{ d}^{-1}$ and an HGR of $1.323 \pm 0.626\% \text{ d}^{-1}$. Meanwhile, the average growth rate on the substrates was 0.410 ± 0.285 and $0.111 \pm 0.392\% \text{ d}^{-1}$ for LGR and HGR, respectively (Figs. 3a-b). However, after 140 and 200 days of experimentation, lower growth rates on the control were observed. The growth of the individuals transplanted on stone substrate had a slight increase from $0.332\% \text{ d}^{-1}$ at 50 days of experimentation to $0.610\% \text{ d}^{-1}$ at 200 days for the LGR and from 0.111 to $0.444\% \text{ d}^{-1}$ for the HGR (Fig. 3a-b). These values were similar to those reported in a field experiment conducted with *Lessonia nigrescens* and *D. incurvata* in central Chile (ca. 33°S). In winter, *D. incurvata* showed an LGR of ca. $0.3\% \text{ d}^{-1}$ and an HGR of $0.4\% \text{ d}^{-1}$, and *L. nigrescens* showed an LGR of ca. $0.9\% \text{ d}^{-1}$ and a HGR of $0.8\% \text{ d}^{-1}$. In spring, kelps increased the growth, *L. nigrescens* reached values of ca. 1.5 and $0.5\% \text{ d}^{-1}$ for LGR and HGR, respectively, and *D. incurvata* showed values of ca. 2 and $1.1\% \text{ d}^{-1}$ for LGR and HGR, respectively (Santelices et al. 1980, Velásquez et al. 2019). The *D. incurvata* growth rates herein reported are concordant with the values observed in autumn and winter, where a low growth for *D. incurvata* is expected.

At the end of the experiment after 200 days, 11 reattached *D. incurvata* juveniles survived (18%), of which six individuals were reattached on the laminar strip device (two per substrates) and five individuals on the pyramid device (three on stones and one on Technyl and polyethylene substrates, respectively). These individuals showed a significant increase in morphological characteristics compared with the initial measures (LM < 0.01 , Figs. 3c-e). Along its range of distribution, coastal populations of *D. incurvata* act as foundation species that can control community structure and ecosystem function (e.g. Castilla & Bustamante 1989, Castilla et al. 2007). Despite the massive *D. incurvata* recruitment in the warm season

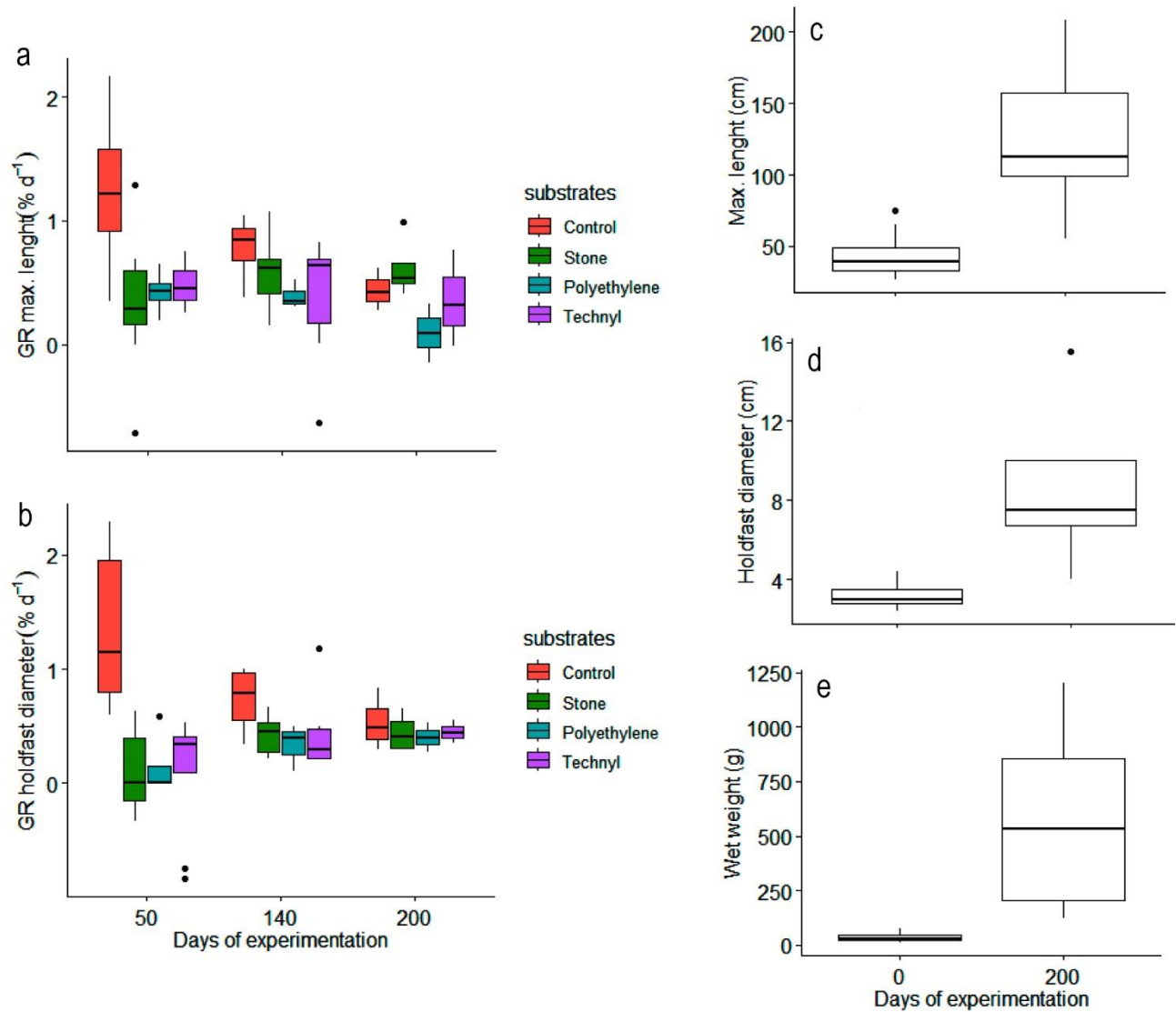


Figure 3. a-b) *Durvillaea incurvata* growth rate (GR) at 50, 140, and 200 days of experimentation; c-e) morphological measures between the experiment's start and end.

(Westermeyer et al. 1994), the individuals are constantly removed by wave action but persist because of a higher colonization rate (Santelices et al. 1980). In this context, the transplantation of *D. incurvata* juveniles settled in zones with high wave action to a sheltered habitat could contribute to population persistence and biomass production.

Furthermore, juvenile transplantation could be used as a technique of stock enhancement intended to increase the natural supply of juveniles, improving the harvest and spawning stock in areas with high exploitation. However, further investigation to improve the restocking techniques is necessary. For instance, the re-attachment of more than one juvenile per substrate

could enhance ecological traits such as the positive effect of coalescence (Santelices et al. 1980, González-Vásquez et al. 2016) and could reduce the maximum loading on individuals and holdfasts under the force of breaking waves (Stevens et al. 2004).

In conclusion, this study demonstrated that transplanted *D. incurvata* juveniles can reattach to the new substrates and increase their size. The juveniles not-reattached and control individuals suffered necrosis, perhaps due to major exposure to high temperature, irradiance, and desiccation stress observed in 2016. Considering economic and technical criteria, the strip device and the stone substrate are recommended for juvenile transplant, supporting the

potential use of transplantation of *D. incurvata* juveniles as a restocking method. However, further research is needed to improve the restocking process, such as reattaching more than one juvenile per substrate and increasing the reattached juveniles' survival.

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