Desiccation resistance and Survivorship of *Caulerpa racemosa* in Galápagos

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This study was conducted under Galapagos National Park Directorate research permit No. PC-12-25. We thank the Galapagos National Park and the Charles Darwin Foundation for their institutional support.

Abstract

Algae are important components of marine ecosystems as they are primary producers and support the aquatic food chain. Invasive species impact ecosystem dynamics by outcompeting native organisms for resources, destroying habitats. These impacts can ultimately lead to a decline in an area's biodiversity. *Caulerpa racemosa* is a cryptogenic (species with an unknown origin) alga displaying invasive characteristics around the Galápagos Marine Reserve (GMR). This alga was first reported around Isabela Island and has since been observed in abundance near Fernandina and Santa Cruz islands, raising concerns about its impact on native reefs. Fragmentation is a key mechanism of spread in *Caulerpa* species, and boat anchors may mechanically fragment and transport *C. racemosa.* While fragmentation and desiccation tolerance are well studied in *C. taxifolia*, they remain understudied in *C. racemosa*. This study investigates the desiccation resistance of *C. racemosa* in Tortuga Bay, Santa Cruz Island, Galápagos, to understand the capabilities of fragments to travel via entanglement with boat anchors between bays and islands. Samples were collected and desiccated for up to three days, then measured daily to assess survivorship and growth. Here we show that desiccation time significantly impacts the growth and survivorship of *C. racemosa*. These results indicate that as desiccation time increases, growth and survivorship decrease. While this is the first time desiccation impacts of *C. racemosa* has been studied in the GMR, similar studies on *C. taxifolia* show these trends. We observed a significant difference in survivorship and mass loss between 0 or 12 hour desiccated fragments and 24 to 72 hour fragments. Our results suggest a potential for anchor spread of *C. racemosa* for short boat trips around the GMR. These results inform management and environmental boating regulations around the GMR. Understanding the desiccation limit and geographic spread potential is crucial for protecting these unique marine ecosystems.

Introduction

The successful establishment of non-native algae is shaped by ecological and environmental barriers. The Galápagos Islands are a group of volcanic islands located ~1,000 km off the coast of continental Ecuador, are geographically isolated, restricting the natural

immigration of new species (Keith et al., 2016). This isolation has led to native organisms evolving with few natural competitors or predators, making them susceptible to invasive species. Thus, while invasive species must overcome physical barriers to dispersal, once introduced, they may thrive in these vulnerable ecosystems due to their ability to utilize resources, fill empty niches, and outcompete native species, thereby disrupting the balance of the ecosystems (Enders et al., 2020; Keith et al., 2016).

Algae, as primary producers, support aquatic food webs and play a crucial role in marine ecosystems (Chapman 2013). Understanding the mechanisms of algal reproduction helps determine whether and how quickly they may expand beyond their current distribution, with implications for the health of the overall ecosystem (e.g., Ceccherelli and Cinelli 1999). One such mechanism, fragmentation, is a type of asexual reproduction where a part of the parent thallus breaks off, travels through the water column, and establishes to grow into an adult clone (Arora and Sahoo, 2015). This reproductive strategy can lead to rapid population growth and widespread distribution (e.g., Ceccherelli and Cinelli, 1999; Ceccherelli and Piazzi, 2005).

Caulerpa racemosa (Forsskål) J. Agardh, is a green alga distributed throughout the tropical and subtropical ocean. It reproduces both sexually and asexually, contributing to its often high population growth in these areas (Ohba et al., 1992). The species has fronds that are four to seven centimeters tall, with oval, trumpet-shaped, or cup-shaped branchlets arranged in clusters in waters up to sixty meters deep (Abbott and Huisman, 2004). While this morphology and depth distribution have been described in other regions, they remain understudied in Galápagos.

Caulerpa racemosa is considered cryptogenic in Galápagos– a species that cannot be resolved as native or non-native (Carlton et al. 2019). (Carlton, 1996). The alga has been present in the Galápagos Islands since at least 1899, and has become increasingly abundant, impacting native marine ecosystem and ecosystem dynamics (Keith et al., 2016; Carlton et al., 2019). First reported around Isabela Island, *C. racemosa* has since been observed near Fernandina and Santa Cruz islands abundant in sheltered bays and mangrove areas (Terán et al., 2025; Keith et al., 2016). This ecological disruption suggests the species is active invasively.

Once established, *C. racemosa* can overgrow native reefs, leading to population decline, disrupting ecosystem processes and the system's overall production (Riegl et al., 2019).

Dispersal through biotic fragmentation, such as by fish grazing, is unlikely as fish are not common grazers of *C. racemosa* due in part to a defensive compound, caulerpenyne, a phototoxic substance that fish avoid (Raniello et al., 2007). However, mechanical fragmentation of *C. racemosa* by boat anchors may help dispersal. Boat anchors dropped into shallow *Caulerpa* dense areas can cut thalli, sending fragments floating through the water column, or collected on anchors, brought onboard, and redistributed at new sites (West et al., 2007). This mechanism has been studied with *Caulerpa taxifolia* (M.Vahl) C.Agardh (West et al., 2007). Fragmentation survivorship of *C. racemosa* under desiccation stress remains largely understudied. However, research shows that some species of *Caulerpa* exhibit short term desiccation resistance, allowing fragments to survive out of water and remain capable of reestablishing (West et al. 2007) Studies on *C. taxifolia* show that fragments can remain viable after two days of desiccation . This supports the idea that fragments can be transported by boat anchors increasing the geographic range of the *Caulerpa* species (West et al., 2007). Understanding this potential dispersal pathway is essential for managing the spread of *C. racemosa* in the Galapápagos Marine Reserve (GMR).

Fragmentation is a key mechanism of spread in *Caulerpa* species, yet while fragmentation and desiccation tolerance are well studied in *C. taxifolia*, they remain largely understudied in *C. racemosa* (Smith & Walters, 1999; West et al., 2007). With its presence and potential ecological impacts in the GMR, understanding possible spread is essential. This study seeks to understand whether desiccation duration influences survivorship and growth in *C. racemosa*. We hypothesize that longer desiccation durations will result in decreased survivorship and growth. As *C. racemosa* continues to expand throughout the GMR, there is a growing concern of potential impacts to the native marine ecosystems. Boat anchors may be able to transport fragments between islands, increasing the species range. This study aims to determine how fragmentation and desiccation resistance of *C. racemosa* contribute to its establishment and expansion within the ecosystems of the GMR.

Methods

Study site and field collections

Caulerpa racemosa samples were collected from rocks of Tortuga Bay, Santa Cruz Island, Galápagos (-0.7610, -90.3323). Sampling was conducted by hand collecting fragments while snorkeling in ~1m depth. Samples were stored in plastic zip top bags with seawater and returned to the wet laboratory at the Charles Darwin Foundation.

Desiccation Trials

Fifty fragments were taken from the bags, cut to a stolon length of 10 cm, cleaned of fouling communities, and weighed on a Joanlab Precision Balance to a resolution of 0.01g. Ten fragments were immediately placed into aquaria using ambient seawater emulating the conditions of Tortuga Bay, each housed within a PVC pipe of 10 cm height and 10 cm diameter. The other 40 fragments were placed onto wax paper and set in a desiccation chamber with the power turned off, emulating a shaded anchor well. Ten fragments were placed into aquaria after the designated time point was reached (i.e., 12, 24, 48, or 72 hours), with each time point assigned to a separate tank. Tanks were filled with ambient seawater to replicate the conditions of Tortuga Bay and were separated by 15 cm PVC pipes. Once in the tanks, wet weight was measured every 24 hours for seven days (Fig. 1). Fragments were taken out, dabbed with a paper towel, weighed on the electronic scale, and placed back into the tank (West et al., 2007). This was repeated twice for a total of 30 fragments tested per desiccation time period. Fragments were considered bleached, defined as those that had lost all pigmentation, a condition previous studies have associated with mortality (West et al., 2007).

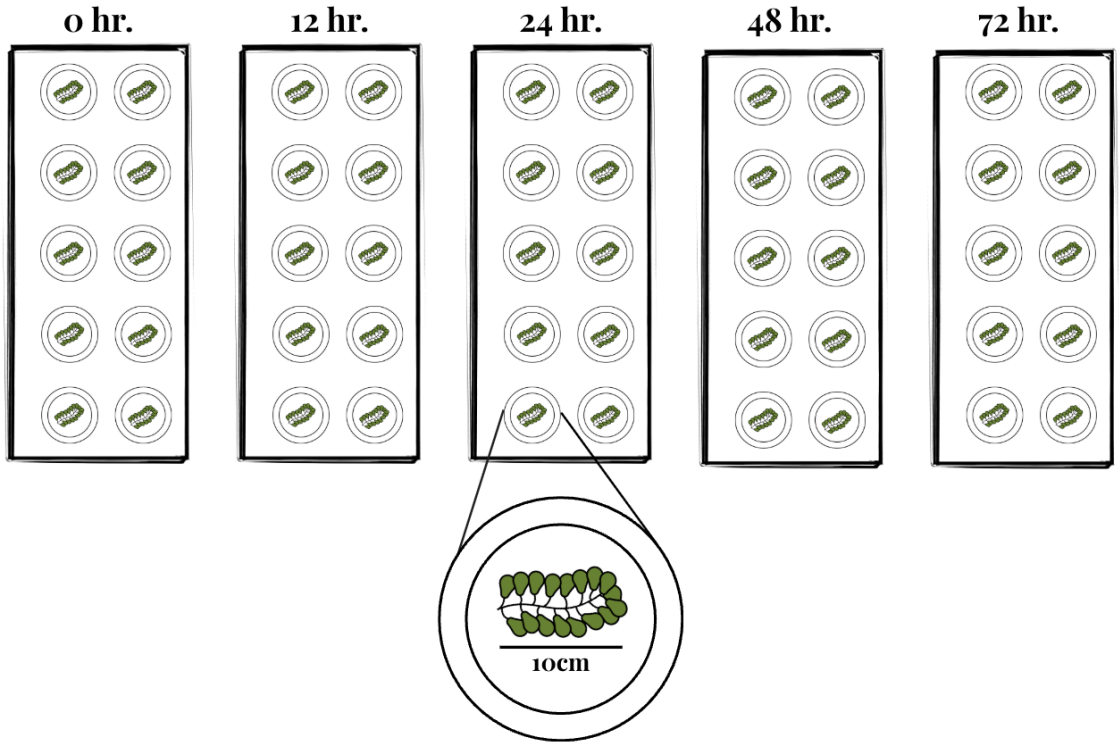

Figure 1. Experimental tank setup used to test desiccation resistance of *C. racemosa*. Each tank contained ten 10 cm fragments placed in individual cut PVC pipes. Tanks are filled with ambient seawater emulating the conditions of Tortuga Bay. Each tank corresponds to a different desiccation duration (i.e., 0, 12, 24, 48, or 72 hours).

Statistical analysis

The time it took for fragments to bleach was compared across desiccation treatments. Following a Shapiro-Wilk test to confirm normality, an ANOVA was conducted to assess differences between the groups (i.e., the desiccation time trials), followed by a post hoc Tukey test for pairwise comparisons.

The initial weight was used as the baseline (time zero), and all subsequent measurements were calculated as changes relative to this starting point. Weight changes were recorded daily for up to 168 hours. No bias in fragment selection was confirmed by testing the start weight between trials using an ANOVA. A line graph, and an ANOVA was conducted following a Shapiro-Wilk test to confirm normality in order to assess differences between time points by trial. To evaluate how weight changed over time and across desiccation treatments, a linear mixed-effects model

was fitted using the lme4 package (Bates et al., 2015), first assessing time and trial as variables and then the interaction between them. An ANOVA was used to test significance between models. Differences between trials were then assessed using an estimated marginal means (least-squares means) with the emmeans package (Lenth, 2025). All analyses were conducted in R (R Core Team 2024).

Results

Fragment Bleaching

From the total 150 fragments (30 per trial), 3, 1, 2, 11, and 15 fragments bleached in the 0, 12, 24, 48, and 72-hour desiccation trials, respectively. Time of bleaching varied significantly across desiccation trials (ANOVA: f=15.87, p=8.46e-11). A post hoc Tukey test revealed significant differences between trials 0 and 72 (p=2.77e-6), 12 and 48 (p=4.75e-2), 12 and 72 (p=5.38e-7), 24 and 72 (p=6.13e-6), and 48 and 72 (p=2.95e-2) (Fig 2.). Increased subfragmentation occurred at higher rates at longer desiccation treatments. Results indicate that *C. racemosa* fragments bleach more quickly as desiccation time increases.

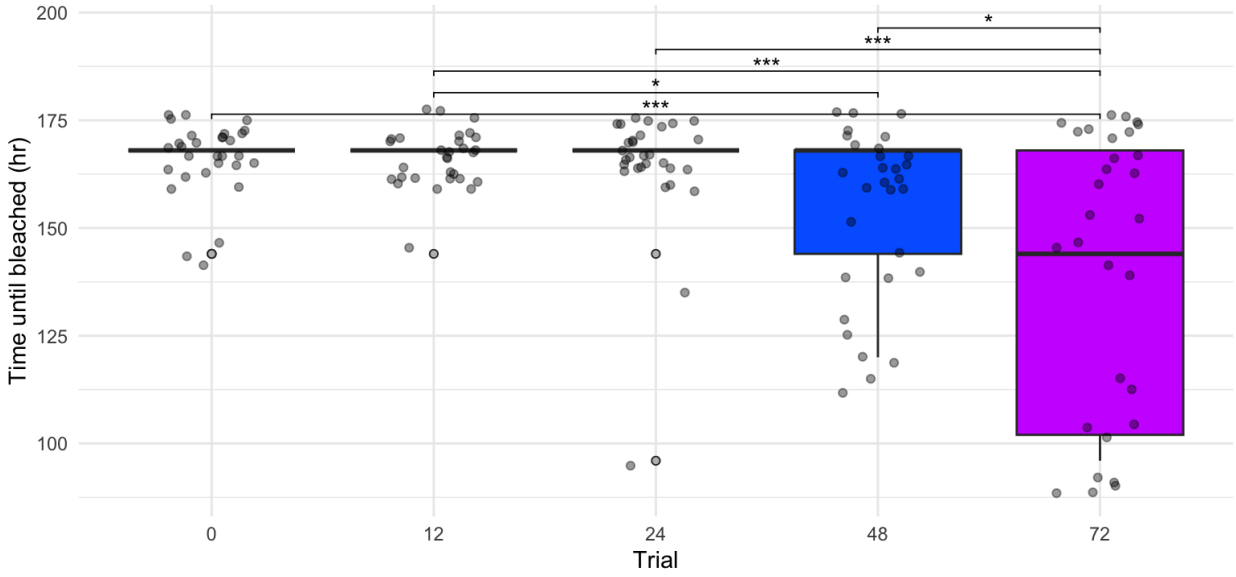

Figure. 2. Hours until *C. racemosa* fragments bleach based on desiccation trial (0, 12, 24, 48, and 72 hours). Each dot represents an individual fragment, grouped by trail.

Weight Change

Weight change from the initial measurement was calculated at each time interval. Starting weight did not vary with desiccation time ($p=0.24$). A line graph revealed significant differences between trials. A linear mixed-effects model revealed a significant interaction between time and trial ($p < 2e\text{-}16$), indicating the relationship between fragment weight and time differed across desiccation treatments (Fig. 3). An estimated marginal means (least-squares means) pairwise comparisons between trials revealed trials 0 and 12 were significantly different from trials 24, 48, and 72 ($p<0.0001$), while no significant differences were found among trials 24, 48, and 72 ($p>0.99$; Table 1). Fragments desiccated for 0 and 12 hours showed minimal weight loss over time, while fragments desiccated for 24, 48, and 72 hours showed significantly greater weight loss. These significant trends and distribution suggest a desiccation limit between 12 and 24 hours.

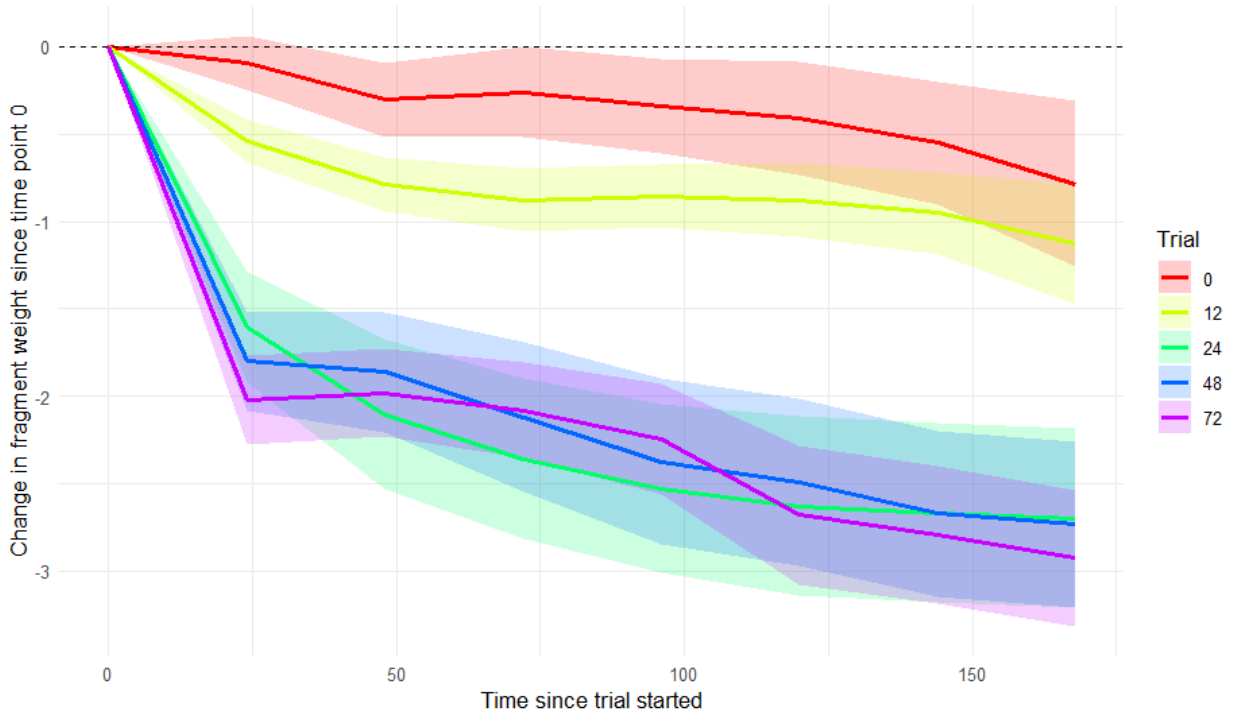

Figure 3. Line Graph showing the change in *C. racemosa* fragment weight in grams relative to the initial measurement over time based on desiccation trials– the number of hours of desiccation. Lines and clouds represent the means with 95% confidence intervals, respectively**.**

Table 1. Pairwise comparisons of estimated marginal means (least-squares means) between desiccation trials using linear mixed effects model p-values. (* indicate significant results ($p<0.05$))

Trial	0	12	24	48	72
0		0.2774	*<0.0001	*<0.0001	*<0.0001
12			*<0.0001	*<0.0001	*<0.0001
24				0.9976	1.0000
48					0.9942

Discussion

This study indicates that desiccation time influences bleaching rates of *C. racemosa,* a proxy for survivorship. The bleaching rates increased as desiccation time increased, showing that 24, 48 and 72 hours bleached at quicker rates. This low desiccation tolerance indicated by bleaching is also shown in *C. taxifolia* with complete fragment death after one day of desiccation (West et al. 2007). Other studies have shown that *C. taxifolia* can withstand up to 10 days of desiccation without high bleaching rates, which may be attributed to genetic differences between geographical groups (Sant et al., 1996; West et al. 2007). It remains a possibility that this desiccation tolerance difference could be found in *C. racemosa* from different geographical locations. While studies on *Caulerpa* are limited, related species and functionally similar aquatic invaders have shown varying patterns of desiccation tolerance. *Codium fragile,* a related species within the order Bryopsidales, has been found to survive on day of desiccation when laid on an exposed deck or anchor well, providing evidence that the species could withstand short out of water trips (Britta, 2003). In contrast, *Cabomba caroliniana,* an invasive submersed macrophyte species in southeast Queensland, Australia, exhibited high desiccation tolerance and survivorship, providing evidence for its potential spread via boat hulls (Schaffelke & Deane, 2014). Our study found that as desiccation treatments increased, fragments exhibit greater subfragmentation, which may have contributed to higher bleaching rates when essential rhizome structures were no longer attached to support regrowth. Our study found that increased desiccation time causes bleaching at a faster rate, which is important for understanding ways in

which the species could spread. This is the first study of desiccation time with *C. racemosa* in Galapagos.

Our study also found that desiccation time has a large influence on the weight of *C. racemosa*. A significant difference in growth was observed between fragments desiccated for 0 and 12 compared to those desiccated for 24 to 72 hours. Previous studies on *C. taxifolia* show that small fragments (~1g) do not survive beyond one day of desiccation, indicating complete mass loss (West et al., 2007). Additionally, *C. taxifolia* fragments exhibit differing rehydration and growth rates based on desiccation duration. One such study shows a sharp decline in rehydration and growth rates of fragments between one hour and 24 hours (Sant et al., 1996). Our study identified two distinct groups - 0 and 12 hours versus 24 to 72 hours - but the absence of an 18 hour desiccation treatment, leaves uncertainty as to whether the desiccation tolerance threshold is closer to 12 or 24 hours. This is the first study to record weight change in relation to desiccation time and suggests potential implications for fragment dispersal.

The results from both bleaching time and weight change indicate that fragments of *C. racemosa* could remain viable during boat trips lasting up to 12 hours. Similar studies on *C. taxifolia* show that fragments can be broken off by anchors and transported in anchor wells, remaining viable for up to one day, indicating spread potential by boats (West et al. 2007). Additionally, *C. racemosa* fragments have been shown to survive up to 10 days of floatation before reattaching, suggesting strong potential for establishment after detachment (Ceccherelli & Piazzi, 2001). While this study did not confirm whether fragments are found in anchor wells, evidence from other *Caulerpa* species and functionally similar aquatic invaders support that possibility (e.g. Sant et al., 1996; West et al. 2007). Although it remains unclear whether fragments can successfully establish after desiccation, the low loss of mass and high survivorship observed up to 12 hours suggests strong potential. This is the first study to assess *C. racemosa* fragment viability for boat spread in the GMR, showing that even after 72 hours of desiccation, some fragments remain viable, with the highest survivorship occurring at 12 hours.

Findings from this study indicate that as desiccation time increases, both bleaching and weight loss of *C. racemosa* fragments increase. Fragments desiccated for up to 12 hours showed low weight loss and high survivorship, suggesting the species can remain viable after a short period out of water. This indicates potential for spread between islands through boat anchors, as

inter-island trips can take as little as three hours. Although *C. racemosa* is considered cryptogenic, its rapid growth, abundance, and ability to overgrow native reefs raise concerns for the biodiversity of the GMR. Management of anchoring practices in high density areas is important to prevent further spread and protect the native biodiversity and ecosystem balance throughout the GMR.

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